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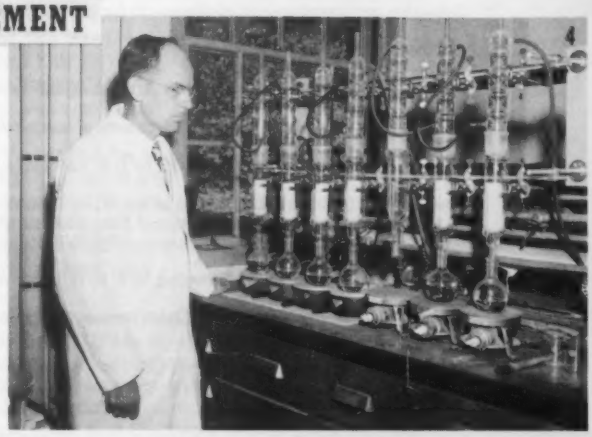

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FISH and WILDLIFE SERVICE
United States Department of the Interior
Washington, D.C.

UNITED STATES
DEPARTMENT OF THE INTERIOR

FRED A. SEATON, SECRETARY

FISH AND WILDLIFE SERVICE

COMMERCIAL FISHERIES REVIEW

A review of developments and news of the fishery industries
prepared in the BRANCH OF COMMERCIAL FISHERIES

A. W. Anderson, Editor

J. Pileggi, Associate Editor H. M. Bearse, Assistant Editor

Mailed free to members of the fishery and allied industries. Address correspondence and requests to the: Director, Fish and Wildlife Service, U. S. Department of the Interior, Washington 25, D.C.

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COMMERCIAL FISHERIES REVIEW

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COLLECTION AND DISTRIBUTION OF MENHADEN OIL FOR RESEARCH USE

By Maurice Bender *

ABSTRACT

More than 900 gallons of menhaden oil were collected and distributed in 15-dram to 55-gallon lots to contractors and other interested groups. Samples represented the entire range of geographical, seasonal, and process-type sources found in the industry. Contractors, whose interests varied from the solution of the most fundamental structural problems to immediate large tonnage applications, were supplied with required oils.

Samples of menhaden oil totaling more than 900 gallons were collected and distributed for research purposes during the 1955 and 1956 menhaden fishing seasons. Contractors and other interested individuals requested quantities ranging from those in 15-dram vials to 55-gallon drums. All these were supplied from either a stock of more than 50 samples kept on inventory or from producers' special stocks. In the latter cases, full histories of the samples were supplied as for those samples collected personally by staff members of the Service's College Park Fishery Technological Laboratory. Oil samples from most of the major menhaden-producing areas were covered in this program. These included the Atlantic Coast south from New York to north Florida and the Gulf Coast from west Florida to Port Arthur, Tex. The samples also represent the entire fishing season from May until January.

Several types of oil processing were represented in these samples. Included were oils removed from the press liquor by centrifuges and then put through polishers for further purification, oils separated by centrifuges alone, and oils gravity separated while held hot in settling tanks. In addition to the oils collected at reduction plants, several samples were obtained from a secondary processor who prepares cold-pressed, kettle-bodied, and alkali-refined oils.

A program was also set up with cooperating commercial oil producers and a research contractor to study certain chemical and physical characteristics of menhaden oils. Samples were supplied on a seasonal and geographical basis. Plants representing the previously-mentioned producing areas as well as the different types of production sent one-pint oil samples at weekly intervals during the entire producing season, which extended from May to January, to the College Park Laboratory. They were then indexed, coded, and shipped to the Contractor at North Carolina State College. Records of these samples were kept at College Park and usually included information about the name and location of the plant, date of processing, time elapsed from catch to landing, gallons of oil per 1,000 fish, and type of separation. The results of these analyses are presently being subjected to statistical treatment in order to determine their significance and allow for more precise interpretation of data.

Menhaden oil samples were sent to a large group of investigators representing many fields of interest. These ranged from research programs of a fundamental

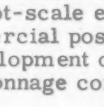
*Bio-Chemist, Middle and South Atlantic Technological Research, Fishery Technological Laboratory, Branch of Commercial Fisheries, U. S. Fish and Wildlife Service, College Park, Md.

nature to those of an immediate practical nature. For example: samples were supplied contractors at the University of Minnesota who were studying the structural analysis of the unsaturated and saturated acids of fish oils by methods designed to eventually concentrate and purify these acids. At the same time members of this same group were studying the possibilities of separating out the fish-oil fatty acids as complexes of the inclusion type with one of the bile acids and with urea. In the area of applied research this group is studying the nature of the chemical reaction involved and the products formed in the development of undesirable odors during storage of fish oils. Results from these researches should greatly increase the range of uses for fish oils. Menhaden oil has also been supplied for a project at the University of Minnesota School of Mines and Metallurgy which, if successfully concluded, might create a large tonnage domestic outlet for fish oils in the flotation separation of metallic ores.

College Park Laboratory staff members were asked to supply a single gallon of menhaden oil to a research group at the Texas A & M Research Foundation for their attempt to develop chromatographic techniques which would be used for qualitative and quantitative analyses of fish oils. However, when it was demonstrated that application of these techniques, which had been developed with pure compounds, to commercially-produced fish oils could not be achieved, the project was terminated. At about the same time more than 30 gallons of menhaden oil were delivered to a research group at the University of Delaware where the nature and properties of the nonglyceride fraction of fish body oils are being studied. These studies require sufficient quantities of oil to separate and purify the nonglyceride fractions which can then be investigated as separate compounds.

Menhaden oil has been supplied for such contrasting areas of experimentation as poultry feeding, leather lubrication, and fungicide activity. At the University of Connecticut feeding studies of high-energy poultry rations containing fish oils were conducted to determine the relative abilities of fish oils and other fats to supply adequately the energy requirements of chickens. The Southern Bio-Research Laboratory at Lakeland, Florida, has reached the pilot-plant stage in their investigation of the use of menhaden oils as fungicides and nematocides for the citrus industry and consequently raised their requirements from 5 gallons to over 100 gallons of oil. The same supply situation recently occurred at the Tanner's Research Council of the University of Cincinnati where pilot-scale experiments were begun with sulfated menhaden oils to develop their commercial possibilities as fat-liquoring agents for leather. Successful commercial development of the latter two uses described above could themselves well require large tonnage consumption of menhaden oil.

It can be readily seen from the foregoing brief review that the areas of research with menhaden oils have been well covered and that the variety of possible collection sources and methods of distribution have also been thoroughly explored and utilized.



TO KEEP NYLON ROPE FROM UNRAVELING

To keep nylon rope from unraveling, encircle the ends with a little tape to keep them in place and then hold them over a burner or torch until they have melted and fused together. When the nylon has cooled, it will harden and the remaining tape can be removed.

--The Progressive Fish-Culturist,
July 1956 (vol. 18, No. 3)
U. S. Fish and Wildlife Service

COMMERCIAL USES FOR MENHADEN OIL

Epoxidation of winterized, deodorized menhaden oil should produce a commercially-attractive plasticizer suitable for blending with other materials into resins, e.g. polyvinyl chloride.

The hydroxylation and epoxidation of menhaden oil as a source of new materials suitable for commercial development were studied by Arthur D. Little, Inc., as part of the over-all Service program to develop new uses for fish oils. Three possible uses for hydroxylated oil were investigated: (1) polyisocyanate adhesives, (2) alkyd resins, and (3) isocyanate foams. Epoxidized oil was investigated as a possible plasticizer for polyvinyl chloride.

Hydroxylated oils showed some possibility for use in adhesive formulations. Hydroxylation converts the double bonds to vicinal glycol structures, the degree of hydroxylation being readily controllable. A typical hydroxylation procedure is given: 288 grams of winterized and deodorized oil was mixed into 123 grams of acetic acid with 6 grams of sulfuric acid. The mixture was heated to 60° C., and 44.8 grams of 50 percent hydrogen peroxide was added slowly, keeping the temperature at 65° ± 5° C. The mixture was held at 65° C. for 4 hours with constant stirring. The reaction mixture was then washed with water, and with a 5-percent sodium carbonate solution. The product was extracted from the mixture with ether. After extraction, the ether solution was dried with magnesium sulfate, and the ether was evaporated from the product. The iodine number dropped from 179 in the raw oil to 127 in the product, indicating a loss of 28.8 percent unsaturation. It was later found that a 20-percent sodium chloride solution in place of plain water and eliminating the alkaline wash speeded up the washing.

The hydroxylated oil was tried in various adhesive formulations, with one using 1-chloro-2, 4-phenylene di-isocyanate (Mondur C) showing some promise. However, though the tensile strength of this adhesive was good, as measured by the sugar-maple block ASTM test D897-49, the impact and shear resistance was poor.

The use of hydroxylated menhaden oils in the formation of alkyd resins and isocyanate foams showed very little promise. The more expensive hydroxylated menhaden oils, due to the added expense of hydroxylation, did not produce better alkyd resins than did the cheaper dehydroxylated vegetable oils used in the resin industry. Hydroxylated menhaden oils formed poor isocyanate foams, having foamed (due to the entrapping of gases within the liquid resin as it sets into the rigid plastic) to only about twice the original volume. A commercially-successful foam will expand 10 to 30 times its original volume.

The most promising results were obtained with epoxidized oils made from winterized and deodorized menhaden oils. Epoxidation was carried out by adding, with agitation, oil to a 10-percent solution of preformed peracetic acid at 20° C. The temperature was allowed to rise to 50° C., and the product was extracted in the same manner as the hydroxylated oil. The report states that peracetic acid for commercial epoxidation of menhaden oil would be formed cheaper in situ--through the action of hydrogen peroxide on acetic acid in the presence of a mineral acid catalyst. (A recent development in epoxidation technique utilizes a cation-exchange resin catalyst.) The chemical cost of epoxidizing menhaden oil using peracetic acid in situ was calculated to be around 12 cents a pound. Acetylated monoglycerides (prepared by transesterification with glycerine and acetylated with acetic anhydride and sodium acetate) were also epoxidized. Acetylation of partially hydroxylated menhaden oil using systems of acetic acid with sodium acetate and acetyl chloride in pyridine proved unsuccessful.

NOTE: ABSTRACT OF FINAL REPORT SUBMITTED BY ARTHUR D. LITTLE, INC., CAMBRIDGE, MASS. THIS FIRM WAS AWARDED A CONTRACT BY THE UNITED STATES FISH AND WILDLIFE SERVICE TO STUDY THE HYDROXYLATION AND EPOXIDATION OF MENHADEN OIL AS A SOURCE OF NEW MATERIALS SUITABLE FOR COMMERCIAL DEVELOPMENT. FUNDS PROVIDED BY THE SALTONSTALL-KENNEDY ACT OF 1954 FINANCED THIS RESEARCH CONTRACT.

Analytical methods employed to measure the degree of hydroxylation and epoxidation are given. The report points out that the iodine number determinations were used as an indirect estimation of the degree of the above reactions. The iodine value gave a measure of the decrease in unsaturation and could not be used as an accurate method of determining the degree of reaction, since cross-linking occurred in the oxidation processes. Therefore, the oxirane or epoxy oxygen and the hydroxyl content was determined. The procedures for these last two determinations are given.

Some important considerations using epoxidized menhaden oil as a plasticizer are as follows: (1) it does not bleed (the tendency of the plasticizer to "bleed" to the surface of the film, thus producing an oily coating); (2) it has low volatility (the length of time a plasticizer remains in the compounded resin without evaporating); (3) it has some decomposition on heating; (4) it appears to be stable towards ultraviolet light; (5) it has no fire-retarding action; (6) its water extraction should be low (the degree of extractibility of the plasticizer from the polymer by water); (7) it has a fishy odor in some samples. Substantially odorless plasticizers were prepared using winterized steam-blown oil. The tendency of creep was not determined. (The lack of "creep" is a property of the plasticizer of imparting flexibility without permitting permanent distortion under stress.)

The report contains 5 tables and 21 references.

NOTE: THIS IS AN ABSTRACT PREPARED BY JOSEPH CARVER, CHEMIST, FISHERY TECHNOLOGICAL LABORATORY, BOSTON, MASS.

WHAT DO WHALES EAT?

The diet of the different kinds of whales is extremely varied, according to the anatomical difference of the various species. The toothed whales (such as the sperm whales, beaked whales, bottlenose dolphin, and killer whale) feed largely upon fishes, squids, and cuttlefish. The killer whale also eat any other mammal found swimming in the water. Bottlenosed dolphins feed upon mullet, sea trout, certain croakers, and even catfish. They have also been reported to eat shrimp. Some of the fresh-water dolphins evidently feed on plant material to some extent.

The baleen whales, of which the blue whale or sulphur bottom (the largest of living animals) is an example, feed upon great masses of very small animals called plankton. In the mouth of this type of whale are suspended the closely set plates of whalebone through the frayed ends of which water is passed while the plankton, such as the shrimplike krill and other forms, are filtered out. The baleen whales feed by cruising with their large mouths wide open, taking in the krill, among others, and occasionally schools of small fishes are also engulfed. One baleen whale was found to contain two tons of plankton in its stomach. Other types of baleen whales are the finback, piked whale, sei whale, and humpback whale.

--"Sea Secrets," May 15, 1956
The Marine Laboratory,
University of Miami,
Coral Gables, Fla.

FISH OIL RESEARCH AT THE HORMEL INSTITUTE

By W. O. Lundberg*

The Hormel Institute is a research branch of the Graduate School of the University of Minnesota, established at Austin, Minn., under an agreement between the University and the Hormel Foundation. It is staffed by academic and civil service personnel of the University. A major portion of the program at the Hormel Institute consists of researches on lipid materials.

Fig. 1 - Main building Hormel Institute of University of Minnesota, Austin, Minn.

Currently, the Hormel Institute is carrying on several projects in the research program started by the U. S. Fish and Wildlife Service with Saltonstall-Kennedy funds. The three original projects begun in 1955 were concerned with (1) determining the structures of fish-oil fatty acids and developing an analytical method for the determination of the fatty acid composition of fish oils, (2) studying the chemistry of the odor problem in fish oils, and (3) studying the fractionation of fish-oil fatty acids by means of "inclusion" compounds. The third one of these projects has been completed and now has been replaced by a fourth project: A study of chemical reactions of fish-oil fatty acids. Although all of the projects fall in the category of what is commonly called fundamental research, it is anticipated that the results will have important practical applications. The following describes each of the projects briefly:

STRUCTURE AND ANALYSIS OF FISH-OIL FATTY ACIDS

For the development of new derivatives and new applications of fish oils, it is obviously important to know the composition and structure of fish-oil fatty acids. Relatively complete information is available about common animal and vegetable fats and oils, but much remains to be learned about fish oils. It is well known that the commercially-important fish oils contain appreciable amounts of highly unsaturated fatty acids and that the carbon skeletons of some of the fatty acids are longer than are those in ordinary animal and vegetable fats, but the locations of the unsaturated centers in the fatty acids have not been established unequivocally. Knowledge of the locations of the double bonds is essential to the development of new and useful derivatives and to the development of analytical methods whereby the composition of fish oils may be determined accurately.

It has been established that the more unsaturated fatty acids of land-animal and vegetable fats have what is called a methylene-interrupted type of unsaturation; that is, a single $-CH_2-$ group lies between successive double bonds in the carbon chain. It is a well-known fact in organic chemistry that a $-CH_2-$ group between two double bonds is highly reactive. The reactivity of the $-CH_2-$ group in unsaturated fatty acids of this type is exploited in the manufacture of commercially-important derivatives of vegetable oils, particularly in the field of protective coatings.

In various past studies of the structure of fish-oil fatty acids, it has been reported that ethylene groups, $-CH_2-CH_2-$, rather than methylene groups, separate the double-bond systems. This type of structure, if it predominates in fish oils, is

*Director of Hormel Institute, University of Minnesota, Austin, Minn.

decidedly disadvantageous insofar as developing certain potentially useful derivatives is concerned, because the ethylene-interrupted type of structure is far less reactive than is the methylene-interrupted type. Further, the existence of any appreciable amount of ethylene-interrupted structures would greatly complicate the problem of analyzing fish oils to determine the proportions of the various types of fatty acids. There was, however, some reason to doubt the reports of the ethylene-interrupted type of structure for fish-oil fatty acids, and thus a critical study of structure was undertaken.

A complete determination of the structure of the more unsaturated fish-oil fatty acids involves the isolation of the individual fatty acids in highly purified form. This has not been accomplished yet in the project at the Hormel Institute, but good progress is being made. Various methods--including solvent segregation, urea-complex fractionation, distillation, and chromatography on silica-gel columns--are being applied in the isolation of individual pure acids.

Even though the pure acids have not yet been isolated, the studies to date have shown that, in the common types of commercially-important fish oils, the more favorable methylene-interrupted type of unsaturation predominates. That at least some of this type of unsaturation is present was evident from the ultraviolet absorption spectra of various fish oils following isomerization with alkali. Alkali-isomerization followed by spectral measurements has served as a basis for the analysis of vegetable oils, and may also be applied to fish oils if methylene-interrupted unsaturation exists.

Fig. 2 - Low-pressure fractional distillation of methyl esters of menhaden oil fatty acids being performed on a Podbielniak Whirling Heli-Band Column at Hormel Institute.

The predominance of methylene-interrupted unsaturation was unequivocally established in studies using soybean lipoxidase. It is well known that this enzyme catalyzes the oxidation only of substances that contain the 1,4 pentadiene grouping, that is, methylene-interrupted unsaturation. In the work at the Hormel Institute, it was found that at least 97 percent (and possibly more) of the polyunsaturated fatty acids of menhaden oil have their double bonds separated by methylene rather than by ethylene groups. On this basis, it appears highly probable that the alkali-isomerization spectrophotometric technique can be adapted to the analysis of common fish oils.

The investigators in this project, Dr. O. S. Privett and his coworkers, are now concentrating on the isolation of individual polyunsaturated fatty acids from menhaden oil for complete structural determination and to establish analytical constants to be used in the analysis of all common fish oils.

CHEMISTRY OF THE ODOR PROBLEM

It is well known that the typical odor of fish oils is a deterrent to their use in many applications. Even though in the production of fish oils the objectionable odor may be removed almost entirely by steam deodorization, an objectionable odor returns in a relatively short time. Evidence indicates that the returning odor may stem from two sources: (1) a liberation of nitrogeneous compounds of low molecular weight from proteins or other nitrogeneous materials of higher molecular weight, and (2) the development of substances of low molecular weight by reaction of atmospheric oxygen with highly unsaturated fatty acids. Obviously it is important to find out what these compounds are and how they are formed, if one is to endeavor intelligently to prevent their formation or to neutralize them as they are formed.

In a first approach to the problem, Dr. J. R. Chipault and his coworkers are studying the products formed in the oxidation of fish oils by air. The volatile odor components are being removed from oxidized oil and analyzed. This is not easy because the offensive volatile components, although possessing powerful odors, ordinarily are present only in very small amounts.

Fig. 3 - Paper chromatography of fatty acids derived from fish oils and their quantitative measurement with a densitometer at Hormel Institute.

Nevertheless, by prolonged and repeated oxidations of relatively large quantities of oils, appreciable amounts of the odor components have been collected. Paper chromatography now is being used to separate various derivatives of the volatile products, and it is hoped that later they may be analyzed more completely by gas-phase chromatography.

The studies to date indicate that the odor and flavor components to a considerable extent consist of unsaturated carbonyl and dicarbonyl compounds derived primarily from the more unsaturated fatty acids. An effort will be made to establish whether the undesirable odors can be attributed predominantly to one category or one type of compound. If this should be the case, there is a good possibility that additives can be found that will react with and destroy the odor components.

SEPARATION OF FATTY ACIDS BY MEANS OF INCLUSION COMPOUNDS

The development of new uses for fish oils depends largely on an exploitation of those structural characteristics of fish oils that distinguish them from common land-animal and vegetable fats and oils. The principal distinguishing characteristics are that fish oils contain appreciable quantities of fatty acids having relatively long carbon-chain lengths and high degrees of unsaturation. For some applications, therefore, it will be important to have economical methods for separating the longer and more unsaturated acids.

Fatty acids have the peculiar property of forming solid complexes or "inclusion" compounds with materials like urea. The extent to which such solid complexes are formed, however, depends upon the structure of the fatty acid. Differences in the tendencies of various fatty acids to form such inclusion compounds may be employed in their separation. Dr. Hermann Schlenk and his coworkers, who have had much experience with inclusion compounds, devoted their efforts to finding materials other than urea that might be employed in such separations. In particular, they studied thiourea and deoxycholic acid. Neither of these "host" compounds would be practical at present because of their cost, even if they were found to give better separations than does urea, but it was important to study them in order to obtain additional information about types of molecules that might be employed effectively.

As is often the case in fundamental research, certain findings were made that were incidental but, nevertheless, important. First, it was found that thiourea was effective in removing peroxides from fish oils, apparently by reaction of the hydroperoxide group with thiourea. This reaction will be explored further in connection with the odor problem, inasmuch as hydroperoxides are probably the precursors of the odor components that develop by oxidation of fish oil. Second, it was found that deoxycholic acid inclusion compounds of fish-oil fatty acids can be separated readily by paper chromatography. This finding will be useful in establishing the purity of the individual fish-oil fatty acids that are being prepared in the first project described

above, and it also has been applied by Dr. Schlenk to show that the content of fatty acids of different chain lengths in menhaden oil are quite different from the value published heretofore.

CHEMICAL REACTIONS OF FISH-OIL FATTY ACIDS

Following completion of the project on inclusion compounds of fish-oil fatty acids, Dr. Schlenk and his coworkers recently have undertaken a new project, the purpose of which is to investigate various types of reactions of fish-oil fatty acids, with a view to developing new derivatives. The project aims to exploit those characteristics of fish oils that cause them to be different from common vegetable and animal fats, notably their content of long-chain highly unsaturated fatty acids.

Reactions of the carboxyl group will not be explored to any appreciable extent because such reactions, and the derivatives obtainable from them, already are well known. Instead, attention will be devoted particularly to reactions of the double bonds. A number of promising reactions have been formulated on paper, and experiments to determine the feasibility of them now are well under way.

SEA LAMPREY

The sea lamprey, Petromyzon marinus, is found along the Atlantic coast of Europe and North America; from the west coast of Greenland to Florida in the western side of the Atlantic; from northern Norway to the Mediterranean and West Africa in the eastern Atlantic. This species runs up fresh rivers to breed and is found in certain American lakes.

Lampreys have on occasion been found fastened to sea fish, and judging from the activities of their landlocked relatives they probably are destructive to some marine species. They have been found preying on mackerel, various herrings, cod, haddock, pollock, salmon, basking sharks, swordfish, hake, sturgeons, and eels. Sometimes as many as 3 or 4 are fast at one time to a single shad.

In the past, considerable numbers of lampreys were caught for food in the rivers of New England, and prior to that in Europe. The lamprey fishery has declined and now only small numbers are taken for consumption.

Another use of the lamprey is to supply biological laboratories with specimens for student use. The larvae are taken in large numbers for bait in certain localities along the Atlantic coast.

--"Sea Secrets," July 17, 1956
The Marine Laboratory,
University of Miami,
Coral Gables, Fla.

CHROMATOGRAPHIC ANALYSIS OF SOME CONSTITUENTS OF MARINE-ANIMAL OILS[1/]

By Raymond Reiser,* Mary Frances Sorrels,** and Maurice Bender***

Most existing methods for the determination of lipid mixtures of natural origin are laborious, time-consuming, and uncertain. This is particularly true in the case of marine-animal oils, which are the most complex of these lipids. This study was undertaken to determine whether the separation and identification of lipid constituents of marine oils could be accomplished with the aid of the silicic acid-impregnated glass-fiber filter paper technique of Dieckert and Reiser. This method allows for the separation of microgram quantities of lipids.

EXPERIMENTAL

Several well characterized lipids were used as reference standards with 10 different solvent systems. R_f values, a measurement of position on the paper which may be used as an identifying index, of the various combinations were determined, each value being the average of at least five tests. Because of the large proportion of triglycerides occurring in marine oils, the mixtures were first separated on a column into 5 groups with 5 solvent systems according to the method of Fillerup and Mead. The eluates were concentrated and then chromatographed on the silicic acid paper.

A certain amount of variation, more pronounced with some of the reference compounds than with others, made absolute R_f values unreliable. For this reason, reference compounds were chromatographed simultaneously with the unknowns. Ascending chromatography was the method used.

The similarity of R_f values for vitamin D_3 and cholesterol in all solvents tried presented a problem which was solved by the addition of iodine to the isooctane ether solvent system. Vitamin D_3 remained at the point of origin while the R_f value for cholesterol was 0.30.

Densitometers were used in an attempt to make the chromatograms quantitative as well as qualitative. Substances such as plastic sprays, collodion, light cedarwood oil, plastic spray over light cedarwood oil, silicone water-repellent spray, and glycerine were used to minimize the irregularity in density in the paper.

RESULTS AND DISCUSSION

Of the five fractions obtained by the column separation, the first three contained all the lipids used in the reference compounds. The fourth contained the fatty acids, and the fifth the phospholipides. This study was not concerned with the fatty acid constituents so the fourth fraction was disregarded. The first fraction contained vitamin A palmitate, cholesteryl palmitate, squalene, hexadecyl palmitate, and the tocopherols. All the triglycerides came through in the second fraction with a part of the aliphatic alcohol. The remainder of the latter appeared in fraction three along with vitamin A alcohol, cholesterol, and vitamin D_3. The 7-dehydrocholesterol, according to preliminary tests, should have appeared in the third fraction. However, it was not recovered from the mixture containing all the reference compounds. The

* PROFESSOR OF BIOCHEMISTRY AND NUTRITION
** RESEARCH ASSISTANT
} DEPT. OF BIOCHEMISTRY AND NUTRITION, RESEARCH FOUNDATION, TEXAS AGRICULTURAL & MECHANICAL COLLEGE.

*** BIOCHEMIST, MIDDLE & SOUTH ATLANTIC TECHNOLOGICAL RESEARCH, FISHERY TECHNOLOGICAL LABORATORY, BRANCH OF COMMERCIAL FISHERIES, U.S. FISH AND WILDLIFE SERVICE, COLLEGE PARK, MD.

1/SUMMARY OF A STUDY CONDUCTED WITH FUNDS PROVIDED BY THE SALTONSTALL-KENNEDY ACT OF 1954 BY CONTRACT WITH THE U. S. FISH AND WILDLIFE SERVICE. THE COMPLETE MANUSCRIPT HAS BEEN SUBMITTED FOR PUBLICATION IN A SCIENTIFIC JOURNAL. THIS IS AN ABSTRACT BY MAURICE BENDER, FISHERY TECHNOLOGICAL LABORATORY, COLLEGE PARK, MD.

fifth fraction contained the phospholipides. In the oil samples studied, phosphatidylethanolamine was the only phospholipide demonstrable. Ninhydrin tests for phosphatidylethanolamine were much less sensitive than the chromatograms. Tests with Dragendorff's reagent for lecithin were negative.

Vitamins A alcohol and D_3 seemed to undergo some change during the process of separation and concentration which made them difficult to demonstrate consistently in the natural oils. "Super D" cod-liver oil showed spots corresponding to triglycerides, vitamin D_3, vitamin A alcohol, phosphatidylethanolamine, and an unidentified component. Silmo cod-liver oil and menhaden oil chromatograms indicated the presence of squalene, triglycerides, vitamins A alcohol and D_3, and phosphatidylethanolamine.

Most of the substances used to rectify the paper for density measurements darkened the paper and were unsatisfactory after drying. Glycerine gave better results than the others but the wet paper was difficult to handle. The lipid spots were too large to be measured with the reflection densitometer. With the transmission densitometer, differences between portions of the paper were often as great or greater than the differences between the paper alone and the charred spots. The spots from a chromatogram were irregular and their variation in size and shape made their densities impossible to measure accurately with this method.

SUMMARY AND CONCLUSION

A scheme for the separation and identification of some constituents of marine animal oils was developed. A preliminary separation on a silicic acid column with five solvent systems was followed by further separation and identification on silicic acid impregnated glass fiber filter paper. This method can be used successfully for qualitative determinations, but the irregularities in the density of the glass paper prevent an accurate quantitative assay.

COMMON MARINE WORMS USED FOR BAIT

The two common marine bait worms are the "sandworm" or "clamworm" and the "bloodworm."

The clamworm (Nereis virens) is usually taken by digging it from its burrow in the muddy or sandy bottom of estuaries during periods of low tide. These burrows vary in depth from a few inches to about eighteen inches or more.

The bloodworm (Glycera dibranchiata) is found in a similar habitat with most of the commercial digging taking place in Maine and extending into Canada.

The greatest use of these worms is in the sport fishery from Maryland to Connecticut with a large demand existing for them in the Long Island Sound area. The worms should be at least 6 or 7 inches long and may be used to catch bluefish, fluke, scup, gray sea trout, sea bass, striped bass, blackfish, kingfish and flounder.

--"Sea Secrets," October 23, 1956
The Marine Laboratory,
University of Miami,
Coral Gables, Fla.

FEEDING FISH OILS TO DOMESTIC ANIMALS

By J. E. Oldfield,* Allen F. Anglemier,* and M. E. Stansby**

BACKGROUND

In recent years, the increasing use of animal fats as major sources of energy in the diets of farm animals has suggested that marine oils might also be used successfully in a similar way. Knowledge of the effects of fish oils in the rations of domestic animals is not nearly as extensive as is that concerning the use of other fishery products.

Two important questions rated priority in the fish-oil feeding investigations. First, the most effective level at which fish oils might be fed should be determined; and second, a comparison of different oils--both from different species of fish and from different processing methods--needed to be made. A program, supported by funds from the Saltonstall-Kennedy Act, was started in July 1955 at Oregon State College to supply information regarding these questions. Experimental work was carried out in the Department of Animal Husbandry, Oregon State College, Corvallis, Ore., and came under the general supervision of the Fishery Technological Laboratory of the U. S. Fish and Wildlife Service at Seattle, Wash.

METHODS AND FACILITIES

Facilities employed in this study included (1) swine from the Berkshire herd of the Oregon Agricultural Experiment Station and (2) individual feeding pens that enabled the keeping of accurate feed and performance records on each animal throughout the test period (figs. 1 and 2). Full-time staff members were responsible for the design of the experiments and the care of the animals. In addition to the facilities of the Department of Animal Husbandry, considerable assistance on this project was made available through the Food Technology Department, Oregon State College (particularly through its branch Seafoods Laboratory at Astoria, Ore.) and the School of Home Economics, Oregon State College.

FIG. 1 - INDIVIDUAL FEEDING PENS IN THE SWINE NUTRITION UNIT AT OREGON STATE COLLEGE MAKE POSSIBLE THE KEEPING OF INDIVIDUAL FEEDING AND PERFORMANCE RECORDS.

Several criteria had to be considered in evaluating fish oils as additives to swine feeds. First, the rate of gain of the animals fed had to be checked to ensure that the oil-containing rations produced at least as rapid gains as did the nonoil-containing rations; second, the efficiency of conversion of the feed to pork had to be satisfactory; and third, the end product of the feeding (hog carcass) had to be acceptable from a market point of view.

Two feeding trials were set up involving 16 pigs each--four pigs were allotted to each ration treatment under consideration. The duration of the trials was from shortly after weaning until the pigs reached about 190 pounds in weight; that is, the animals were carried through the normal growing-fattening period followed in farm practice.

* DEPARTMENT OF ANIMAL HUSBANDRY, OREGON STATE COLLEGE, CORVALLIS, ORE.
** CHIEF, PACIFIC COAST AND ALASKA TECHNOLOGICAL RESEARCH, FISHERY TECHNOLOGICAL LABORATORY, BRANCH OF COMMERCIAL FISHERIES, U. S. FISH AND WILDLIFE SERVICE, SEATTLE, WASH.

In the first trial, California sardine oil replaced 5-, 10-, and 15-percent of the grain portion of the diets. In addition, a nonoil-containing diet was fed as a check control. At the conclusion of the tests, all the animals were slaughtered at a commercial packing house, carcass measurements were made, and samples were obtained. A shoulder roast was cut out of each carcass and, after being cooked under standard controlled conditions, was subjected to taste testing by a panel.

FIG. 2 - FEEDING FACILITIES IN INDIVIDUAL SWINE PENS KEEP FEED SEPARATE FOR EACH ANIMAL. COMMON WATER TROUGHS ARE PROVIDED.

After the results of the first trial were made known, a second was started using different oils fed at a constant level. In the second trial, the oils in each case made up 5 percent of the total diet. Again, a nonoil-containing diet was fed as a check. The oils used in this case were derived from menhaden. One sample was crude menhaden oil, one was alkali-refined-and-bleached menhaden oil, and the third was polymerized (kettle-bodied) oil. These rations were fed over the same length of time, and the results were evaluated in the same way as already described in the first experiment.

PROGRESS MADE TO DATE

In the first trial, it was found that the pigs fed 5 or 10 percent California sardine oil grew at about the same rate and at about the same feed efficiency as did those that got the check ration. When oil replaced 15 percent of the grain in the diet, the pigs went off feed and failed to grow properly. When the carcasses were evaluated, it was found that a fishy odor and taste were present, especially in the fat, of all pigs fed all three levels of fish oil. In the case of the pigs on the 10-percent and 15-percent level of oil substitution, a marked yellow color was evident in the fat, which further detracted from the quality of the carcass.

It was concluded from this trial that a 5-percent level of fish-oil addition should be satisfactory in swine rations provided that something could be done to the oil to prevent the "fishiness" in the carcass.

In the second trial, it was found that pigs fed 5 percent of either crude or alkali-washed menhaden oils gained weight at a better rate than did the pigs on the control ration, while those fed the polymerized menhaden oil gained weight less quickly than did the controls.

The carcass quality of the pigs fed the polymerized oil, however, was vastly superior to that of the pigs fed either of the other types of oil and closely approximated that of the controls. From this trial, it was concluded that the type of oil fed is most important in determining the effectiveness of that oil in livestock rations. It seems possible that a fish oil polymerized under carefully controlled conditions might yield a product satisfactory from the nutritional (that is, growth-promoting) as well as the carcass-quality standpoint.

NEW PRODUCTS FROM FISH OILS

Part III - Preparation and Separation of Fatty Alcohols Made from Fish Oils--A Preliminary Study

By Edward H. Gruger, Jr.*

ABSTRACT

Sodium reduction of methyl palmitate, methyl stearate, methyl oleate, and of the natural glycerides of menhaden and pilchard oils was carried out in order to prepare the corresponding long-chain fatty alcohols. The resulting prepared fatty alcohols were then fractionated into saturated and unsaturated alcohols by acetone crystallization at low temperature. A complex mixture of unsaturated alcohols prepared from pilchard oil was used in studies of the efficiency of various separation methods. The methods studied were fractional distillation, urea countercurrent distribution, and column chromatography. Ultraviolet spectrophotometry was used to measure qualitatively the degree of unsaturation in the various fatty-alcohol fractions.

INTRODUCTION

A project has been undertaken at the Seattle Technological Laboratory to prepare long-chain unsaturated fatty alcohols from fish oils and to study the corresponding physical properties of these alcohols, with the ultimate objective of providing information that will stimulate the development of new fish-oil products (Stansby 1956).

Why study fatty alcohols? The fatty alcohols are extremely valuable in organic research because of the wide variety of other substances that can be prepared from them. They are among the most important of the synthetic fatty derivatives (Ralston 1948) that can be prepared from fatty acids and esters.

Some of the reactions of primary aliphatic alcohols are esterfication, oxidation or catalytic dehydrogenation to aldehydes, dehydration to unsaturated hydrocarbons, and halogenation to alkyl halides. The alkyl halides thus formed from fatty alcohols also act as important intermediates in the synthesis of other compounds. The formation of various esters, 1/ aldehydes, and alkyl halides offers the greatest potentialities for fatty alcohols with respect to chemical synthesis of new products or the modification of older established products.

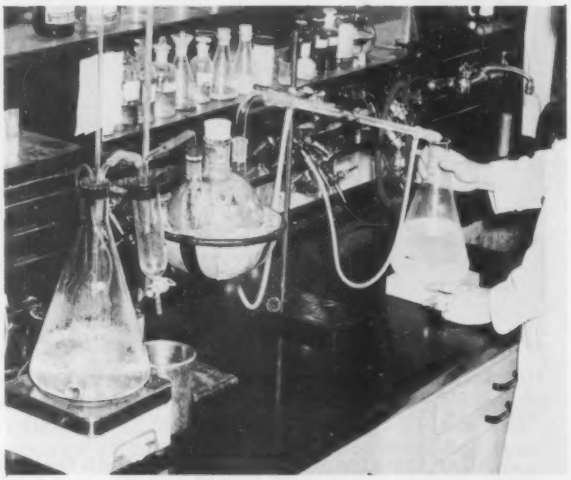


FIG. 1 - HYDROLYSING A SODIUM-REDUCTION-MIXTURE OF FISH OILS IN THE PREPARATION OF LONG-CHAIN FATTY ALCOHOLS.

Owing to the fact that unsaturated alcohols of high molecular weight are difficult to prepare in a state of high purity, few reliable constants for such compounds have been reported in the literature (Ralston 1948). The initial work at the Seattle laboratory therefore has been to obtain information regarding a satisfactory method of isolating pure polyunsaturated fatty alcohols and to report in the scientific literature

* ORGANIC CHEMIST, FISHERY TECHNOLOGICAL LABORATORY, BRANCH OF COMMERCIAL FISHERIES, U. S. FISH AND WILDLIFE SERVICE, SEATTLE, WASH.

1/ FOR EXAMPLE $R'COOR$, WHERE R IS THE PORTION DERIVED FROM THE LONG-CHAIN FATTY ALCOHOL.

NOTE; "PART 1 - INTRODUCTION" APPEARED IN THE AUGUST 1956 (P. 1) ISSUE AND PART 11 - POLYAMINO FATTY ACIDS DERIVED FROM FISH OILS" IN THE SEPTEMBER 1956 (P. 7) ISSUE OF COMMERCIAL FISHERIES REVIEW.

reliable constants for these alcohols. The techniques commonly employed for separating fatty acids and their derivatives are as follows: (1) fractional distillation (Weitkamp 1955), (2) fractional solvent crystallization (Brown 1955), (3) countercurrent distribution (Dutton 1955), (4) chromatography (Allen 1955), and (5) fractionation by the formation of inclusion compounds (Schlenk 1954). The research work here has employed all of these techniques.

EXPERIMENTAL RESULTS AND DISCUSSION

PREPARATION OF FATTY ALCOHOLS: To prepare long-chain fatty alcohols from fish oil, we used a sodium reduction process (Hansley 1947; Pryde 1951). The method is as follows: To a rapidly-stirred suspension of 92 grams (4.00 moles) of finely divided sodium, in 100 milliliters of refluxing xylene, is slowly added a mixture of 300 grams of fatty acid glycerides, 264 milliliters (214 grams, 2.10 moles) of 4-methyl-2-pentanol (the reducing alcohol), and 600 milliliters of xylene. After the reaction of fatty-acid glycerides with the sodium and reducing alcohol is complete the resulting mixture is hydrolyzed.

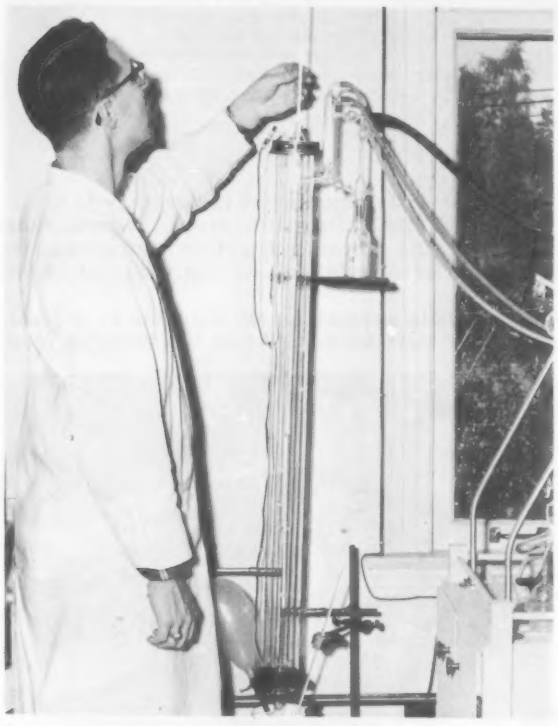


FIG. 2 - FRACTIONAL DISTILLATION APPARATUS WITH PROVISION FOR INTERNAL-INERT ATMOSPHERE THROUGH THE USE OF AN ATTACHED NITROGEN-FILLED BALLOON.

The hydrolysis apparatus used (fig. 1) provides for simultaneous steam distillation of the xylene and the 4-methyl-2-pentanol. At the start of the hydrolysis, 300 milliliters of boiling water is added to a 3-liter, three-neck "hydrolysing flask." Steam, from a steam generator, is passed through the hot water via a gas-inlet tube attached to one of the side arms of the hydrolysing flask. The steam generator is fitted with a water-vapor trap between it and the gas-inlet tube. To the other side arm of the hydrolysing flask is connected a Liebig-type condenser. At the end of the condenser is a flask to receive the condensed xylene and reducing alcohol.

After the steam has passed through the hydrolysing flask for 3 minutes, the sodium-reduction mixture is added through the center neck of the flask, by the use of a large funnel-tube that extends beneath the surface of the hot water. The joint at the center-neck of the flask is tightly sealed to prevent air leakage. The long-chain fatty alcohols, liberated during the hydrolysis, are recovered from the hydrolysis-mixture by an ethyl ether-petroleum ether extraction.

Experience with the details of the sodium-reduction process was gained through the preparation of cetyl alcohol (1-hexadecanol) from methyl palmitate, octadecyl alcohol (1-octadecanol) from methyl stearate, and oleyl alcohol (9-octadecen-1-ol) from methyl oleate. The process was then applied to the natural glycerides of menhaden and

pilchard oils. With the latter oils, the crude mixtures of saturated and unsaturated fatty alcohols were obtained as light-brown, mildly viscous liquids in yields of 85 to 90 percent. The saturated and unsaturated fractions were separated by crystallization in acetone at -18° C. It was the unsaturated fraction, obtained in the foregone manner, that became the starting material for further studies of the separation and the identification of unsaturated fatty alcohols.

FRACTIONAL DISTILLATIONS: A sample of mixed unsaturated fatty alcohols (prepared from pilchard oil) was used to study the effects of fractional distillation. The distillation apparatus (fig. 2) included an electrically-heated column jacket for simulating adiabatic conditions, a still-head with internal reflux condenser and adjustable take-off for controlling the rate of distillate take-off for a given reflux ratio (usually about 8 to 1), and a pressure regulator for maintaining a constant pressure during distillations.

The distillations were carried out at a pressure of 0.1 to 10.0 millimeters of mercury. A considerable amount of decomposition and polymerization occurred during the course of a distillation, thereby decreasing the yield of the more highly unsaturated fatty alcohols. The data in table 1 show iodine numbers and refractive indices of the distilled fractions. The yield of total distillate indicated that a large amount of high boiling, polyunsaturated alcohols were not recovered. Unrecovered pot residue amounted to as much as 34.3 percent of the starting material for one of the best distillations.

Table 1 - Analytical Data for Alcohol Fractions Obtained by Fractional Distillation

Fraction 1/	Boiling Point		Refractive Index	Iodine Number	Hydroxyl Equivalent 2/
	Temperature	Pressure			
No.	°C.	Mm. Hg.	n_D^{30}		
1	113.0-126.0	0.50	1.4458	26.6	234.6
3	139.0-141.0	0.50	1.4562	104.0	252.8
9	163.0-165.0	0.51	1.4620	132.5	277.2
11	167.0-170.0	0.63	1.4630	134.2	290.0
12	170.0-172.0	0.50	1.4611	131.4	285.0
17	187.0-189.0	0.60	1.4843	198.2	311.8
18	189.0-191.0	0.65	1.4899	216.4	311.7
21	204.0-208.0	0.65	1.4960	199.8	329.9
22	208.0-210.0	0.65	1.4980	222.9	348.3

1/THESE ARE THE MAJOR FRACTIONS THAT APPEARED TO CONTAIN NEARLY PURE COMPOUNDS.
2/THEORETICAL MOLECULAR WEIGHTS OF SATURATED ALCOHOLS ARE AS FOLLOWS: C_{16}, 242; C_{18}, 270; C_{20}, 298; C_{22}, 326; C_{24}, 354.

Oxygen-free nitrogen was bubbled through the pot charge while the distillation proceeded, in order to lessen the loss due to polymerization. This bubbling was done by the use of a nitrogen-filled balloon attached to a capillary tube (fig. 2) inserted into the distilling flask containing the charge of unsaturated fatty alcohols. Additional work along these lines is postponed until distillations can be studied on a spinning-band type of distillation column such as the type described by Murray (1951).

UREA COUNTERCURRENT DISTRIBUTION: In another phase of the research, a study was made of the utility of fractionation by the use of urea inclusion compounds incorporated into a countercurrent distribution system (Domart, Miyauchi, and Sumerwell 1955). It was shown that the urea-countercurrent-distribution technique makes possible the separation of highly unsaturated alcohols. To what extent this separation is complete has not yet been determined for mixtures occurring naturally. The experiments performed thus far have shown an abnormal weight-distribution curve for urea countercurrent distribution of the unsaturated alcohols prepared from pilchard

oil. Nevertheless, refractive indices of the fractions obtained have indicated that a separation was made. The fractions separated in this manner are currently being studied as to the degree of unsaturation and the chain-length of the respective alcohols.

CHROMATOGRAPHIC SEPARATIONS: The alcohols obtained from fractional distillation have appeared colorless, whereas those obtained from the urea countercurrent distribution have appeared yellowish. Studies on column chromatography were started to determine to what extent the various fractions separated by these other methods could be further purified by the removal of polymerized materials. The chromatography has been carried out principally with fractions from the countercurrent distribution.

Preliminary work was undertaken to find an adsorbent that would remove the impurities and still permit good yields of the purified fractions. A mixture of Fuller's earth, powdered silica, and powdered calcium oxide was found to give fractions much lighter in color. Inasmuch as the fractions going into or coming out of the column were not protected by an inert atmosphere, it is believed that this adsorbent will be more satisfactory when used with nitrogen.

DETECTION OF UNSATURATION: Ultraviolet spectrophotometry has been used to determine the extent of conjugated and nonconjugated double bonds in the polyunsaturated alcohols being studied. The ultraviolet spectra is being used to determine the success in isolating the more highly unsaturated fatty alcohols by the various separation techniques. Alkali isomerization of nonconjugated unsaturation is employed with ultraviolet absorption when the number of double bonds in the alcohols of the particular fractions is being measured. 2/

A certain amount of conjugation has taken place during the sodium-reduction process, as the ultraviolet spectra of the crude alcohol mixtures have indicated. This was expected, since the method used to isomerize and measure the nonconjugated double bonds involves treatment of the unsaturated alcohols with strong alkali 2/ at a high temperature (180° C.) and since the unsaturated portions of the prepared alcohols are in contact with a strongly alkaline mixture at a temperature close to the boiling point (138° C.) of the xylene solvent during the sodium reduction process. The amount of isomerization and possible decomposition that occurs during this treatment can be minimized by working rapidly and by utilizing an inert atmosphere over the reaction mixture. To obtain the alcohols that correspond to the naturally-occurring fatty acid glycerides, we will have to develop a method for the removal of the conjugated unsaturated alcohols that occur via the sodium-reduction process and/or other reactions.

Abu-Nasr and Holman (1954) have pointed out that conjugation of unsaturated-fatty-acid esters occurs during fractional distillation. They therefore suggested that fractional distillation be avoided as a final step in purifying polyunsaturated esters. This suggestion would apply also to the polyunsaturated alcohols. For this reason, the detection of conjugated unsaturation in fractions of distilled unsaturated alcohols is not surprising. It is also expected that such analytical data as iodine numbers will be erroneously low 3/ when quantities of conjugated unsaturated alcohols are present in the various fractions.

It has been indicated here that double bonds are conjugated by an alkaline treatment during sodium reduction and by fractional distillation. Thus, it can be seen why difficulties may be encountered in isolating pure fractions of nonconjugated polyunsaturated fatty alcohols. Displacement chromatography is one method of isolating pure compounds of this type (Abu-Nasr and Holman 1954). Another method that has

2/ AMERICAN OIL CHEMISTS' SOCIETY TENTATIVE METHOD CD 7-48.
3/ IODINE IS VERY SLOW TO REACT WITH CONJUGATED DOUBLE BONDS; THEREFORE EXPERIMENTALLY-DETERMINED IODINE NUMBERS ARE LOW WHEN THE CONVENTIONAL ANALYTICAL METHODS ARE USED.

proven successful for the separation of fatty materials is the formation of urea inclusion compounds (Schlenk 1954); (Schlenk and Holman 1950); (Abu-Nasr, Potts, and Holman 1954). Both of these techniques are to be given additional study at this laboratory.

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NEW PRODUCTS FROM FISH OILS

Part IV - Preliminary Investigations on Alkyl Halides, Silicones, and Quaternary Ammonium Salts

By Edward H. Gruger, Jr.*

ABSTRACT

LONG-CHAIN ALKYL HALIDES WERE PREPARED FROM PURE CETYL ALCOHOL, N-OCTADECYL ALCOHOL, AND OLEYL ALCOHOL. SOME ALKYL HALIDES WERE PREPARED FROM ALCOHOLS DERIVED FROM MENHADEN AND PILCHARD OILS. THESE COMPOUNDS WERE USED TO PREPARE SILICONES AND QUATERNARY AMMONIUM SALTS.

THE SILICONES PREPARED RESULTED FROM THE HYDROLYSIS AND SUBSEQUENT DEHYDRATION OF CHLOROSILANES OF THE TYPE $RSiCl_3$. THESE ALKYLTRICHLOROSILANES WERE PRODUCTS OF ALKYL MAGNESIUM HALIDE REACTIONS WITH SILICON TETRACHLORIDE.

THE QUATERNARY AMMONIUM SALTS ARE A COMBINATION OF THE ALKYL HALIDES AND TRIETHYLAMINE. THE LONG-CHAIN, POLYUNSATURATED ALKYLTRIETHYLAMMONIUM BROMIDES FROM FISH OIL ARE BEING TESTED AS ORE-FLOTATION COLLECTORS AND AS FUNGICIDES FOR THE CITRUS INDUSTRY.

INTRODUCTION

There is great need for research and development of derivatives [1/] prepared from fish-oil fatty acids. In the past, relatively little work has been done on these derivatives as compared with that done on the derivatives from the fatty acids of vegetable and animal oils.

Fig. 1 - Ultraviolet absorption measurements being made on long-chain fatty alcohols used to prepare alkyl halides.

Fish oils are known to be unique in containing a high percentage of long-chain fatty acids with many double bonds. These unique properties of fish oils make them potentially valuable for the manufacture of many industrial and pharmaceutical products (Stansby 1956). The present paper is one in a series reporting the research now being carried out at the Seattle Technological Laboratory on these potential products from fish oils.

Polyunsaturated fatty alcohols (Gruger 1956) have been prepared from fish oils and are being used as the starting material in the preparation of other derivatives. Polyamino fatty acids (McDonald and Gruger 1956), alkyl halides, silicones, and quaternary ammonium salts are presently the compounds being studied in the research program.

ALKYL HALIDES: The alkyl halides prepared from fish oils are the products of direct reaction of the fatty alcohols with an appropriate halogenating agent. Special

*ORGANIC CHEMIST, FISHERY TECHNOLOGICAL LABORATORY, BRANCH OF COMMERCIAL FISHERIES, U. S. FISH AND WILDLIFE SERVICE, SEATTLE, WASH.

1/THE TERM DERIVATIVE IS USED IN ITS BROADEST SENSE. THUS, ANY GROUP OF COMPOUNDS THAT CAN BE PREPARED FROM FATTY ACIDS, EITHER DIRECTLY OR INDIRECTLY, IS CONSIDERED AS A FATTY-ACID DERIVATIVE.

NOTE: "PART I - INTRODUCTION" APPEARED IN THE AUGUST 1956 (P. 1) ISSUE; "PART II - POLYAMINO FATTY ACIDS DERIVED FROM FISH OILS" IN SEPTEMBER 1956 (P. 7) ISSUE; AND "PART III - PREPARATION AND SEPARATION OF FATTY ALCOHOLS MADE FROM FISH OILS--A PRELIMINARY STUDY" IN THIS ISSUE (P. 13) OF COMMERCIAL FISHERIES REVIEW.

attention is being given to the long-chain polyunsaturated alkyl halides, although the preliminary work has been centered on saturated compounds that are commercially available in a relatively high state of purity.

Alkyl halides have numerous applications. Employed in various coupling reactions they are used to prepare long-chain hydrocarbons. Alkyl magnesium halides--commonly called Grignard reagents--offer a means of producing many useful compounds of distinct chemical structures, such as silicones, primary alcohols, substituted secondary and tertiary alcohols, ketones, and sulfinic acids. Alkyl halides are also useful in undergoing displacement reactions to form such compounds as nitriles, ethers, and mercaptans. Each one of these products prepared from the alkyl halides have great potential utility.

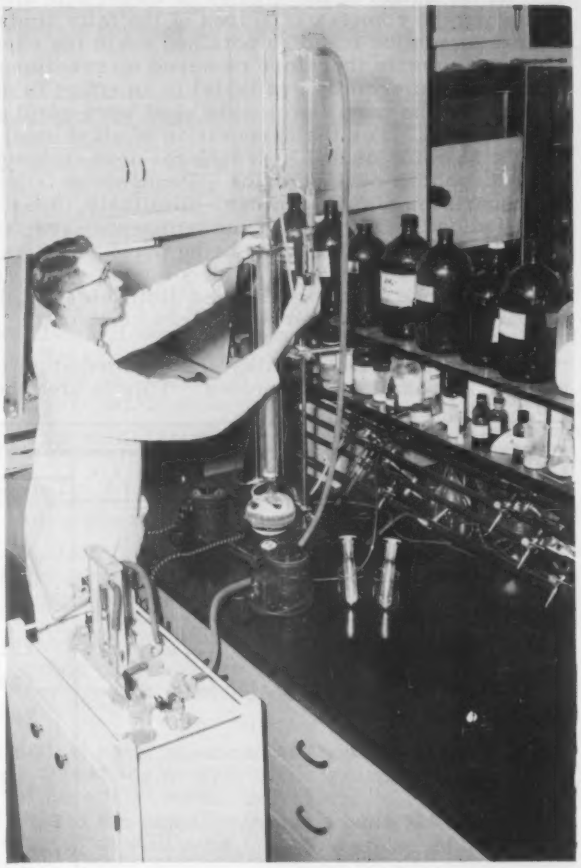

Fig. 2 - Fractional distillation of long-chain alkyl bromides prepared from fatty acids of fish oils.

SILICONES: Another field of investigation has been the silicones. McGregor (1954) defines a silicone as "a compound containing the elements of silicon, oxygen and organic groups, the silicon being present in sufficient amount to affect the properties measurably." Silicones are known to be quite stable at high temperatures, to be somewhat resistant to oxidation, and to have good lubricating and water-repellent properties. Silicones containing long-chain polyunsaturated alkyl groups would probably possess special properties and would have interesting potential uses. There is a possibility that a highly stable drying oil or a tough resilient copolymer could result from this type of silicone.

QUATERNARY AMMONIUM SALTS: During the work on the alkyl halides, a satisfactory derivative was sought for use in the identification of the various long-chain alkyl halides that were being synthesized. Quaternary ammonium salts were chosen because of their relative ease of formation and reasonably sharp melting points. (With these compounds, a sharp melting point, however, does not necessarily indicate purity.) The quaternary ammonium salts have recently become commercially important as germicides and as surface-active agents in the textile field (Harwood 1952). Ralston (1948) has suggested their use in the production of disinfecting and preserving agents, detergents, fire-extinguishing foams, wetting agents, and flotation agents. Therefore the quaternary ammonium salts were studied somewhat extensively, with a view toward their eventual use with the unsaturated alkyl halides prepared from fish oils.

PRELIMINARY INVESTIGATIONS OF LONG-CHAIN ALKYL HALIDES

At the outset of the studies of long-chain alkyl halides, it was quickly learned that when the complex mixtures of the fatty acids found in fish oil were used, very little information could be obtained about the chemistry of the substances reacting. The experiments therefore centered on reactions of single, pure, long-chain compounds (commercially available) in an effort to study the completeness of a given reaction. The pure compounds used were cetyl alcohol, n-octadecyl alcohol, and oleyl alcohol. For the preparation of alkyl chlorides, the halogenating agents used were zinc chloride and hydrochloric acid, thionyl chloride, phosphorus trichloride, and phosphorus pentachloride. Phosphorus tribromide was reacted with the alcohols to prepare the alkyl bromides. Similarly, hydriodic acid was used to prepare the alkyl iodides. Of the chlorinating agents used, thionyl chloride gave the best yield. When the alkyl halides were being purified by fractional distillation, the alkyl iodides showed a marked tendency to decompose. The decomposition was made evident by the appearance of iodine-colored distillates. Table 1 lists the halogen compounds prepared and their observed physical constants.

The relative ease of alkyl-halide formation was observed to be in the order: iodides > bromides > chlorides. This is also the order of reactivity of the corresponding alkyl halides (Fieser and Fieser 1944).[2/] For the purpose of carrying out laboratory-scale experiments with alkyl halides, the bromides are most often chosen, since they are more reactive than are the chlorides and more economical to work with than are the iodides. Therefore, as the experiments in the Seattle laboratory progress with alkyl halides prepared from fish oil, only the bromides will be made. If any larger-than-laboratory-size quantities are made, the alkyl chlorides will probably be used, since they are cheaper to prepare than are the bromides.

Table 1 - Physical Constants for Alkyl Halides

Compound	Boiling Point		Refractive Index
	Temperature	Pressure	
	°C.	Mm.Hg	n_D^T
Cetyl chloride	191-194	15	-
Cetyl bromide	197-203	21	1.4593^{20}
Cetyl iodide	151-153	3	$1.4389^{49.9}$
n-Octadecyl chloride	195-203	12	-
n-Octadecyl bromide	213-216	14	$1.4441^{49.9}$
n-Octadecyl iodide	180-190	7	-
Oleyl chloride	153-163	3	1.4582^{20}
Oleyl bromide	141.7-147.7	1.0	1.4692^{25}
Satd. alkyl bromides 1/	170.5-185.0	4.0	1.4619^{24}
Unsatd. alkyl bromides 2/	181.0 201.0	9 0.57	1.4832^{25}

1/ Prepared from menhaden oil. 2/ Prepared from pilchard oil with boiling points at two different pressures.

INVESTIGATION OF SILICONES DERIVED FROM FISH OIL

There are four commercial methods for preparing silicon compounds containing carbon. These methods are (1) the "direct process," (2) the Grignard method, (3) the olefin addition method, and (4) the sodium method (McGregor 1954). The Grignard

2/ FOR A GIVEN ALKYL RADICAL, R, THE ORDER OF REACTIVITY OF ALKYL HALIDES IS RI > RBR > RCL. THEREFORE, THIS WOULD BE TRUE FOR ANY GIVEN LONG-CHAIN ALKYL HALIDE.

method is the one that has been studied at the Seattle laboratory. This method involves the substitution of an organic group for one or more of the chlorine atoms in silicon tetrachloride. In this way, compounds of the types $RSiCl_3$, R_2SiCl_2, R_3SiCl or R_4Si can be formed from $SiCl_4$, where R represents a group, such as the octadecyl group ($-CH_2(CH_2)_{16}CH_3$).

Table 2 - Analytical Data for Alkyl Trichlorosilanes

Alkyl Trichlorosilane ($RSiCl_3$)	Boiling Point		Amount of Silicon in $RSiCl_3$	
	Temperature	Pressure	Experimental	Theoretical
	°C.	Mm. Hg	Percent	Percent
Dodecyl trichlorosilane . .	154.0-157.0	10.0	35.32	35.01
Cetyl trichlorosilane . . .	199.0-201.0	10.0	27.21	27.56
Oleyl trichlorosilane . . .	166.0-167.0	1.5	29.63	29.55

GRIGNARD METHOD: In the Grignard method, the Grignard reagent (alkyl magnesium halide) is first prepared. This reagent is made by placing magnesium chips and an appropriate dry solvent--usually ethyl ether--into a reaction vessel equipped with a stirrer and reflux condenser. Next, an alkyl halide (the long-chain alkyl bromides, in the present case), diluted in the same solvent, is allowed to drip into the stirred mixture of magnesium and solvent, while protecting the reaction mixture from the moisture in the air. This reaction is represented by the following general equation (where X represents the halogen):

$$RX + Mg \xrightarrow[\text{dry ether}]{} RMgX$$

The next step in the synthesis involves the reaction of the Grignard reagent and silicon tetrachloride. The Grignard reagent is added, with stirring, to the silicon tetrachloride in dry ether. This reaction is represented as follows:

$$RMgX + SiCl_4 \longrightarrow RSiCl_3 + MgXCl$$

It is evident from the equation that, by varying the ratio of amounts of Grignard reagent to silicon tetrachloride, the other types of compounds (R_2SiCl_2, R_3SiCl, and R_4Si) may be formed.

ALKYL CHLOROSILANES: The alkyl chlorosilanes, as they are called, have a marked reactivity toward compounds containing a hydroxylic group or groups. Therefore, by reacting alkyl chlorosilanes with water, for example, one can form compounds called alkyl silanols. (This is the reaction that has been investigated most in the Seattle laboratory in the study of silicones.) Because low-molecular weight alkyl silanols dehydrate readily to form silicon polymers or silicones, it was decided that this same reaction should be tried in the preparation of long-chain alkyl silanols. One type of reaction involved can be expressed, generally, as follows:

$$RSiCl_3 + 3H_2O \longrightarrow RSi(OH)_3 + 3HCl$$

$$xRSi(OH)_3 \longrightarrow \left[\begin{array}{c} R \quad R \\ | \quad | \\ -O-Si-O-Si-O- \\ | \quad | \\ O \quad O \\ | \quad | \\ -O-Si-O- \\ | \\ R \end{array}\right]_x + xH_2O$$

(a resinous cross-linked polymer)

Thus far, the only type of alkyl chlorosilane that has been produced in the Seattle laboratory is of the $RSiCl_3$ type (table 2). The type R_2SiCl_2 was attempted; however, the analyses indicated that only one mole of Grignard reagent reacted instead of two moles, in all the chlorosilanes made here. Apparently, there is too much steric hindrance in the $RSiCl_3$ formed for it to react with a second mole of the Grignard reagent. In any case, more severe reaction conditions are necessary.

REACTIVITY OF ALKYL HALIDES: Another phase of the silicone work included a study of the reactivity of the alkyl halides in the preparation of the corresponding Grignard reagents. The findings were in accord with the order of reactivity given earlier (RI > RBr > RC1). In some attempts, the long-chain alkyl chlorides would not react with the magnesium, even under forcing conditions. It therefore appears that the yield of Grignard reagent and consequently the yield of silanol is dependent on the reactivity of the alkyl halide.

Table 3 - Silicon Content of Silicones

Alkyl Group, R	Amount of Silicon Found	Theoretical Amount of Silicon	
		$[R_2SiO]_x$ 1/	$[RSiO_2]_x$ 2/
	(Percent)		
n-Butyl	23.8	17.7	23.9
n-Hexyl	18.4	13.1	19.3
n-Decyl	9.36	8.58	13.9
n-Dodecyl	9.61	7.33	12.2
n-Hexadecyl	6.58	5.14	9.83
n-Octadecyl	4.59	3.66	6.65
Oleyl	2.43	3.68	6.68

1/ A POLYMER UNIT RESULTING FROM $R_2Si(OH)_2$ DEHYDRATION.
2/ A POLYMER UNIT RESULTING FROM $RSi(OH)_3$ DEHYDRATION.

HOMOLOGS OF THE SATURATED ALKYL SERIES: In attempting to purify silanols or silicones containing long-chain saturated alkyl groups, we learned that no common solvent would completely dissolve them. The dehydration products of cetyl silanetriol and n-octadecyl silanetriol, in particular, were only partially soluble. These latter compounds are white solids, whereas the homologs of lower molecular weight are liquids.

Table 4 - Melting Points of Triethylalkylammonium Halides

Alkyl Halides Reacted With Triethylamine	Melting Point of the Salt (Uncorrected)
	°C.
Cetyl bromide	173.0-176.5
Cetyl iodide	182.0-182.5
n-Octadecyl bromide	178.0-179.5
n-Octadecyl iodide	184-187
1,10-Diiodooctadecane 1/	117.0-119.0
Satd. menhaden bromides	69-75
Oleyl bromide	243-245
Unsatd. pilchard bromides	135 2/

1/ 1,10-DIIODOOCTADECANE WAS PREPARED FROM OLEYL ALCOHOL BY THE REACTION OF TWO MOLES OF HI PER MOLE OF THE ALCOHOL. A SUITABLE SOLVENT GIVES MOSTLY THE ONE ISOMER.
2/ THESE SALTS WERE GREASES; TEMPERATURE GIVEN IS THAT AT WHICH THE GREASE BECAME FLUID.

At one point in the investigation, it was thought that the compounds produced in this manner were the products of a major side reaction. A study was made of the formation of various homologs of a saturated alkyl series to determine if the technique used produced solids in all cases. The corresponding derivatives were prepared from n-butyl bromide, n-hexyl bromide, n-decyl bromide, n-dodecyl bromide, and n-hexadecyl (cetyl) bromide. The silicon content of the resulting silicones are listed in table 3. Using the same reaction conditions for each preparation, we observed that a transition from liquid to solid product occurred between the C_{10} and C_{12} alkyl-containing derivatives. It was assumed that the formation of solid products was due to molecular weight characteristics and not to side reactions.

Some preliminary work was done in preparing silicones from saturated and unsaturated alcohols (via the halides) prepared from menhaden oil. Further work has been postponed until the problems characteristic of the reaction are worked out for the pure unsaturated compounds. The only preparation of a pure unsaturated alkyl-containing silicone that has been attempted is a derivative of oleyl bromide. In contrast to the C_{18} saturated derivative, the oleyl or C_{18} unsaturated derivative is a slightly viscous liquid. This observation indicates the possibility of forming similar polyunsaturated derivatives from fish oil.

QUATERNARY AMMONIUM SALTS PREPARED FROM FISH OILS

In the preparation of a quaternary ammonium salt, an alkyl halide is reacted with an appropriate amine. The following equation is representative:

$$RX + R'_3N \longrightarrow R'_3N.RX$$

Triethylamine quaternary salts have been prepared from the alkyl halides listed in table 4.

Harwood (1952) mentions that melting points of quaternary ammonium compounds are not a good criterion for purity because the compounds are subject to decomposition when heated to the melting point. Some uncorrected, observed melting points are listed in table 4. Apparently, the observations for the triethylamine salts of oleyl bromide and n-octadecyl bromide are anomalous, since it would not be expected that the melting point of the oleyl bromide derivative would be higher than that of the n-octadecyl bromide derivative.

Samples of some quaternary ammonium compounds prepared were tested for fungicidal activity and for use as ore-flotation collectors. The fungicidal activity was shown by workers at Florida Southern College [3] to be positive for the quaternary ammonium iodides prepared from an unsaturated fraction of menhaden oil. This salt, however, was prepared only in a small amount at a time, so that when a request was received for additional samples for testing, only the unsaturated alkyl bromides from pilchard oil were available for synthesis. There should be little difference, however, between the two types of compounds. Results of these tests on the pilchard oil derivative are still pending. The quaternary ammonium salts of unsaturated pilchard bromides are being tested as ore-flotation collectors at the University of Minnesota. [4] The results of these tests are also pending.

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UTILIZATION OF FISH OILS IN ORE FLOTATION

By S. R. B. Cooke* and M. E. Stansby**

ABSTRACT

RESEARCH IS BEING CONDUCTED TO RETURN FISH OILS TO THEIR PREVIOUS USE AS FLOTATION AGENTS FOR THE SEPARATION OF METALS FROM ORES. POTENTIAL FLOTATION USAGE OF SUCH OILS AT ONE-HALF POUND PER TON OF IRON ORE IS ABOUT 5,000,000 GALLONS A YEAR. STUDIES HAVE BEEN CONDUCTED ON THE EFFECTS OF CONCENTRATION, FATTY ACID CARBON-CHAIN LENGTH, AND UPON THE DEGREE OF UNSATURATION OF SUCH COMPOUNDS ON THEIR EFFICIENCY AS FLOTATION AGENTS. FATTY ACIDS WITH IODINE VALUES OF 70-115, INDICATING MODERATE UNSATURATION, HAVE GIVEN THE GREATEST EFFICIENCY. SEPARATION OF THE INDIVIDUAL FATTY ACIDS FROM THE OILS HAS BEEN COMPLETED AND WORK ON THE EFFECTS OF CARBON-CHAIN LENGTHS ON FLOTATION EFFICIENCY IS IN PREPARATION.

Although at one time fish oils were used to a small extent in ore flotation, this is no longer the case. Research carried out by competing oil producers has resulted in the development of oil derivatives such as alkyl amines, that in some cases are superior to the aliphatic acids, although oleic acid is still widely employed as a flotation collector.

FIG. 1 - H. S. CHOI USING A FAGERGREN FLOTATION CELL TO INVESTIGATE THE USE OF FISH OIL FATTY ACIDS IN ORE CONCENTRATION. THE HEAVY GLASS CELL CONTAINS AN AQUEOUS PULP OF FINELY-GROUND IRON ORE. FATTY ACIDS SELECTIVELY COAT THE IRON OXIDE MINERAL PARTICLES, MAKING THEM HYDROPHOBIC. MECHANICAL STIRRING OF THE PULP AND SIMULTANEOUS ADMISSION OF AIR PERMITS THE COATED IRON OXIDE PARTICLES TO BECOME ATTACHED TO AIR BUBBLES, WHICH RISE TO THE SURFACE AND ARE SCRAPED INTO THE PAN IN FRONT OF THE OPERATOR, THUS PRODUCING AN IRON-RICH "CONCENTRATE." THE SILICEOUS GANGUE MINERALS, BEING HYDROPHILIC, REMAIN IN THE PULP AND DO NOT CONTAMINATE THE "CONCENTRATE," THUS PRODUCING A "TAILING."

The flotation process is currently employed for sulfide ores (using sulphydric collectors), as well as in many applications to other types of ore. The flotation process can be applied to the concentration of low-grade iron ores. At the present time, the high-grade iron ores are nearing exhaustion, and these lower-grade ores, which are very abundant, will be the principal future source of iron production in this country. The School of Mines and Metallurgy and the Mines Experiment Station at the University of Minnesota have carried out research for the past 30 years to develop methods for concentrating these ores. Based on this research, plants valued at over 500 million dollars are now under construction.

There are two general types of iron-concentration methods that can be used and that are being considered at the present time. One of these involves magnetic separation; the other flotation. Although the magnetic-separation process has been widely investigated and most of the plants now under construction make use of this method, there are many iron ores to which the magnetic-separation process is not directly applicable and for which flotation may well be the favored method. Should the flotation process eventually be adopted for this purpose there would be a very great expansion in demand for flotation agents. Less than one-half pound of oil or oil derivative is required for flotation of a ton of iron ore, but since nearly 100 million tons of iron ore are processed annually, the amount of oil required for the flotation of this amount of ore is considerable (about 5 million gallons).

* PROFESSOR OF MINERAL DRESSING AND METALLURGY, SCHOOL OF MINES AND METALLURGY, UNIVERSITY OF MINNESOTA, MINNEAPOLIS, MINN.

* * CHIEF, PACIFIC COAST AND ALASKA TECHNOLOGICAL RESEARCH, FISHERY TECHNOLOGICAL LABORATORY, BRANCH OF COMMERCIAL FISHERIES, U. S. FISH AND WILDLIFE SERVICE, SEATTLE, WASH.

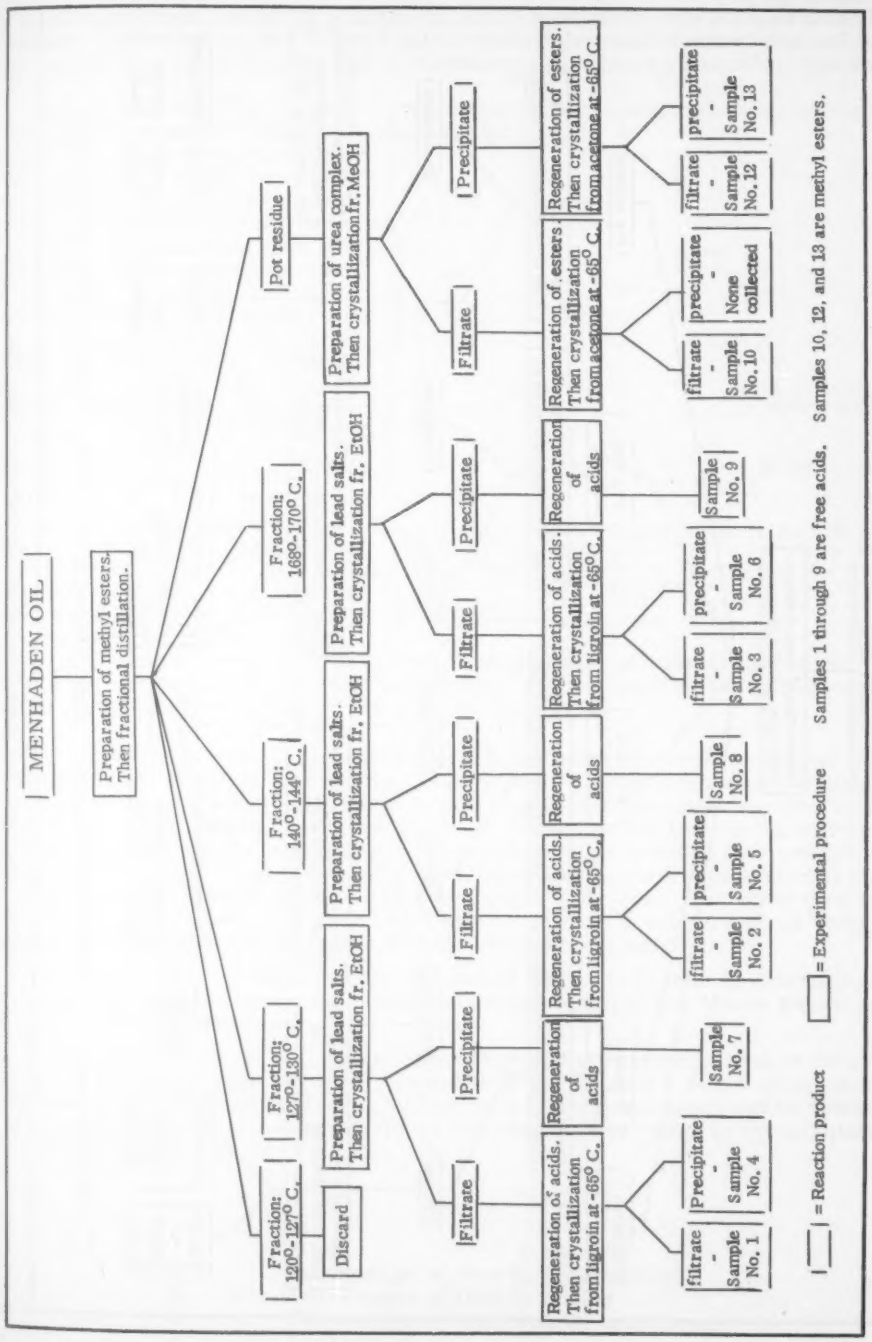
MENHADEN OIL
Preparation of methyl esters. Then fractional distillation.
Fraction: 120°-127° C.
Fraction: 127°-130° C.
Fraction: 140°-144° C.
Fraction: 168°-170° C.
Pot residue
Discard
Preparation of lead salts. Then crystallization fr. EtOH
Preparation of lead salts. Then crystallization fr. EtOH
Preparation of lead salts. Then crystallization fr. EtOH
Preparation of urea complex. Then crystallization fr. MeOH
Filtrate
Precipitate
Filtrate
Precipitate
Filtrate
Precipitate
Filtrate
Precipitate
Regeneration of acids. Then crystallization from ligroin at -65° C.
Regeneration of acids
Regeneration of acids. Then crystallization from ligroin at -65° C.
Regeneration of acids
Regeneration of acids. Then crystallization from ligroin at -65° C.
Regeneration of acids
Regeneration of esters. Then crystallization from acetone at -65° C.
Regeneration of esters. Then crystallization from acetone at -65° C.
filtrate - Sample No. 1
Precipitate - Sample No. 4
Sample No. 7
filtrate - Sample No. 2
precipitate - Sample No. 5
Sample No. 8
filtrate - Sample No. 3
precipitate - Sample No. 6
Sample No. 9
filtrate - Sample No. 10
precipitate - None collected
filtrate - Sample No. 12
precipitate - Sample No. 13
= Reaction product
= Experimental procedure
Samples 1 through 9 are free acids.
Samples 10, 12, and 13 are methyl esters.

FIG. 2 - FLOW SHEET FOR SEPARATION OF THE COMPONENTS OF MENHADEN OIL.

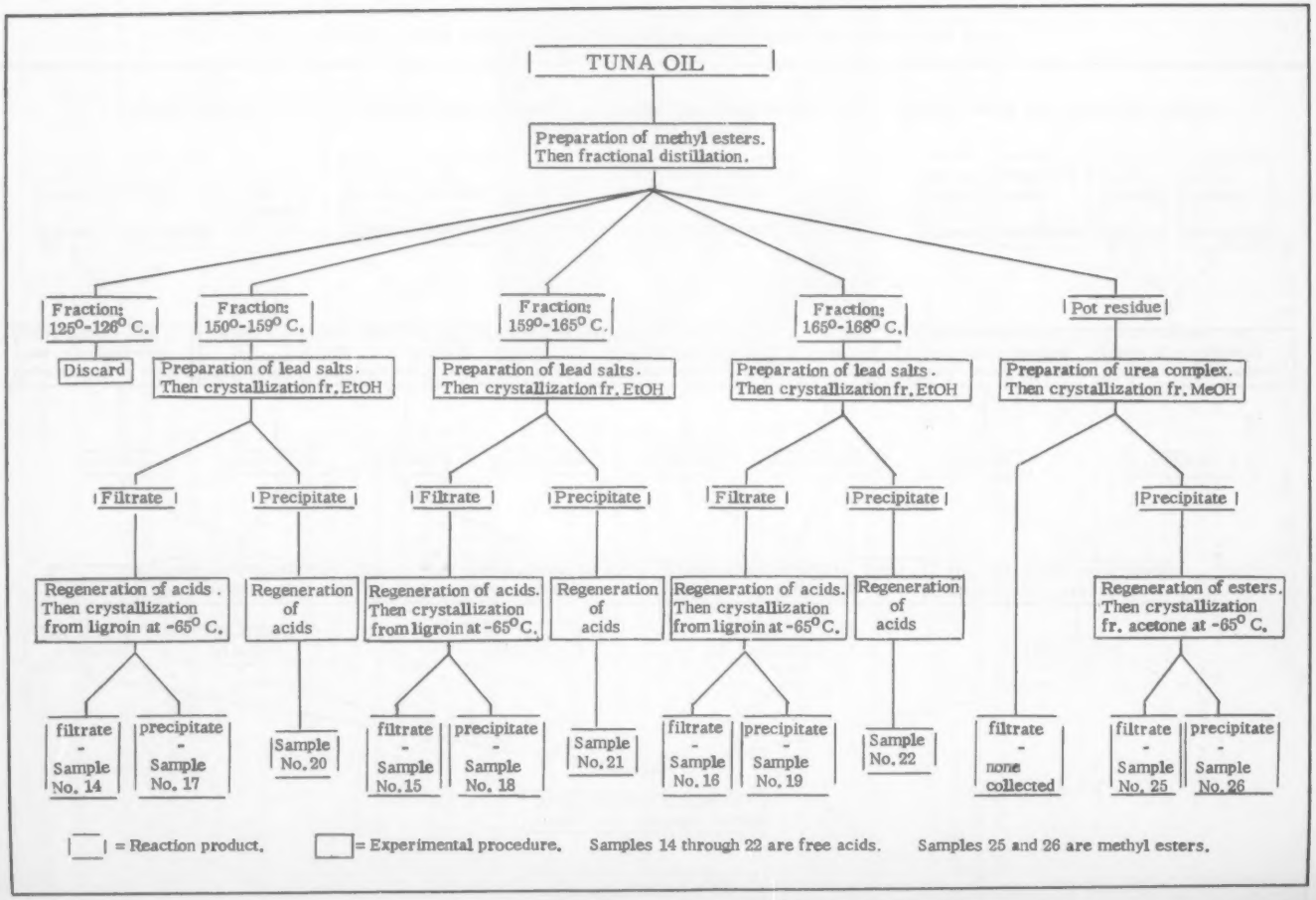
TUNA OIL
Preparation of methyl esters. Then fractional distillation.
Fraction: 125°-126° C.
Fraction: 150°-159° C.
Fraction: 159°-165° C.
Fraction: 165°-168° C.
Pot residue
Discard
Preparation of lead salts. Then crystallization fr. EtOH
Preparation of lead salts. Then crystallization fr. EtOH
Preparation of lead salts. Then crystallization fr. EtOH
Preparation of urea complex. Then crystallization fr. MeOH
Filtrate
Precipitate
Filtrate
Precipitate
Filtrate
Precipitate
Precipitate
Regeneration of acids. Then crystallization from ligroin at -65° C.
Regeneration of acids
Regeneration of acids. Then crystallization from ligroin at -65° C.
Regeneration of acids
Regeneration of acids. Then crystallization from ligroin at -65° C.
Regeneration of acids
Regeneration of esters. Then crystallization fr. acetone at -65° C.
filtrate - Sample No. 14
precipitate - Sample No. 17
Sample No. 20
filtrate - Sample No. 15
precipitate - Sample No. 18
Sample No. 21
filtrate - Sample No. 16
precipitate - Sample No. 19
Sample No. 22
filtrate - none collected
filtrate - Sample No. 25
precipitate - Sample No. 26
= Reaction product.
= Experimental procedure.
Samples 14 through 22 are free acids.
Samples 25 and 26 are methyl esters.

FIG. 3 - FLOW SHEET FOR SEPARATION OF THE COMPONENTS OF TUNA OIL.

In addition to this very large potential use of oils for the concentration of iron ore, there are sizable quantities of other ores that at present are being concentrated by flotation methods, including manganese and nonmetallic ores such as fluorite and phosphate. The work on this project will consider the application of fish-oil derivatives to iron-ore concentration and to flotation of manganese and other types of ores.

Research on the effect of the chain length and degree of unsaturation of such compounds on their efficiency as ore-flotation agents has been carried out at the School of Mines and Metallurgy of the University of Minnesota. The present study includes an extension of this work to consider the longer-chain compounds of higher degree of unsaturation contained in fish oils to determine if they offer any advantages over the flotation agents being currently employed. Some of the preliminary work on shorter-chain fatty acids already carried out at Minnesota has indicated that there are advantages both to long-chain length and to high degree of unsaturation. In past work, alkyl amines in some cases have proved superior to the fatty acids as flotation agents. In the initial experimental work, tests have been confined to use of fish-oil fatty acids. Some tests of derivatives of fish oil, such as alcohols, will be made to see if they will function as compatible frothers with the fish-oil fatty acids.

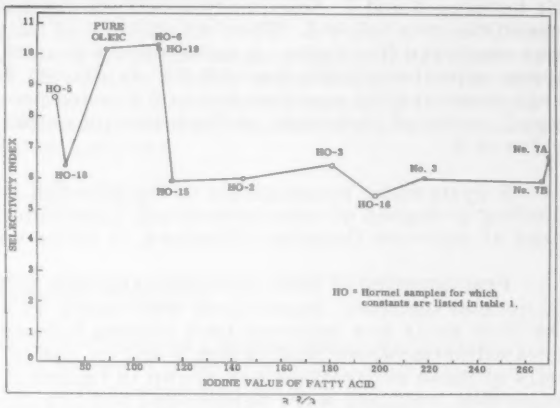

FIG. 4 - RELATIONSHIP BETWEEN THE EFFICIENCY OF THE VARIOUS FATTY ACID FRACTIONS IN THE COLLECTION OF ORE AND THEIR DEGREE OF UNSATURATION AS MEASURED BY IODINE VALUE. ORE WAS CONDITIONED WITH FATTY ACIDS AT 60 PERCENT SOLIDS, FLOATED AT 19-PERCENT SOLIDS AT PH 6, AND ROUGHER CONCENTRATES WERE CLEANED TWICE.

During the first year of the investigation, the work has been restricted entirely to studies on the flotation of iron ore. Most of the work has been with Michigan specularite (jasper ore), which at present is being commercially concentrated by a flotation process. A standard procedure has been developed for testing the efficiency of different flotation agents with this ore. The ore first is crushed fine enough to pass through a 20-mesh screen and then is de-slimed (that is, very fine material of minus 10-micron size is removed) by use of a hydraulic elutriator. Flotation then is carried out using each fatty acid under test at a series of pH values and, in some instances, employing different ratios of fatty acid to ore (figure 1).

The iron content of the concentrates and of the tailings then is determined. This work has been carried out in the analytical laboratories of the Mines Experiment Station, University of Minnesota.

In calculating results, one finds that both the final content of iron in the concentrates and the percentage recovery of iron is of importance. For a given series of tests, the curves obtained by plotting both of these factors against pH can be compared. A more simple measure of the efficiency of collectors is possible by calculation of the selectivity index (S. I.):

$$S.I. = \sqrt{\frac{M}{m} \times \frac{n}{N}} \quad \text{where}$$

M = Percentage of iron in concentrate
m = Percentage of iron in tailing
N = Percentage of insoluble in concentrate
n = Percentage of insoluble in tailing

The selectivity index varies from 1, when no concentration has been made, to infinity for complete concentration (never practically obtainable).

Concentration experiments have been completed at a series of pH values between 5 and 9, comparing the selective index when using oleic acid and the mixed fatty acids from pilchard oil. On the ores so far investigated, the optimum pH seems to lie between 6 and 7. Selectivity is extremely poor at pH 8 or higher, and frothing is unsatisfactory below 5. When an addition of fatty acid of one pound per ton of ore was employed (the optimum value for oleic acid), the pilchard fatty acids showed a lower selectivity index than did the oleic acid. When, however, the pilchard-oil fatty acid concentration was lowered to 0.5 pound per ton of ore, the selectivity index was equal to that of oleic acid, at the lower pH values, and was markedly higher, at a pH value of 8.

A systematic investigation of the effect of fish-oil fatty acids, fractionated according to degree of unsaturation and approximate chain length upon their effectiveness as iron-ore flotation collectors, is under way.

Fractionation of both menhaden and tuna oils was carried out in the laboratories of Hormel Institute. Separations were made by distillation of the methyl esters of the fatty acids in a spinning-band column followed by lead-salt and low-temperature crystallization from Skellysolve F and by use of urea-complex separations. The details of these separations are shown in figures 2 and 3. Chemical constants of the separated fractions were determined and are shown in table 1.

Table 1 - Fractions of Fatty Acids or Methyl Esters Obtained from Menhaden and Tuna Oils

Menhaden Oil				Tuna Oil			
Sample No.	Quantity	Saponification Value	Iodine No.	Sample No.	Quantity	Saponification Value	Iodine No.
	Ml.				Ml.		
1	5	1/	1/	14	10	1/	1/
2	100	214.5	144.0	15	150	194.1	115.8
3	150	207.2	181.2	16	50	193.6	197.4
4	10	1/	1/	17	10	1/	1/
5	250	219.2	67.6	18	150	200.8	71.2
6	250	204.1	109.7	19	150	197.2	109.4
7		2/	2/	20		2/	2/
8		2/	2/	21		2/	2/
9		2/	2/	22		2/	2/
10	225	166.5	196.5				
12	50	179.7	202.2	25	25	175.8	149.8
13	50	175.2	111.1	26	75	170.8	69.7

1/ DENOTES SAMPLE TOO SMALL FOR ANALYSIS.
2/ DENOTES SOLID, SATURATED FRACTION AND THAT NO ANALYSIS WAS MADE.

Application of these various fractions of fish oils to the flotation of certain Mesabi Range wash-ore tailings are now under way. Although all fractions have not been completely tested, enough results for some preliminary conclusions have been obtained. Figure 4 shows the relationship between the degree of unsaturation of the various fractions, as expressed by iodine value, and their efficiency as ore collectors, as given by the selective index. These preliminary results indicate that the efficiency of fatty acids as collectors for iron ore is determined, at least in part, by the degree of unsaturation. Fatty acids having iodine values in the range of 70-115 give the greatest efficiency. This range includes the degree of unsaturation of oleic acid, which has been used as a standard in the work to date.

The oleic acid used has been a relatively pure product, whereas in the commercial flotation operation, an impure commercial grade oleic acid is employed. In some

preliminary experiments, it was found that the efficiency of oleic acid was greatly affected by its purity. Accordingly, work is now under way to compare the efficiency of the oleic acid used in commercial iron ore flotation with that of the purer oleic acid used in this research. Work also will be started soon on derivatives of fish-oil fatty acids prepared at the Service's Seattle Fishery Technological Laboratory.

ACKNOWLEDGMENT

To H. S. Choi, who carried out the ore flotation tests; to K. V. Batra, who carried out the fatty acid separations; to Dr. W. O. Lundberg and staff of the Hormel Institute, who carried out the complex fractionation separations; and to H. H. Wade, Acting Director of the University of Minnesota Mines Experiment Station, whose analytical laboratories carried out the iron analyses.

FERTILIZING THE SEA

In different parts of the world, the technique of applying chemical fertilizers to increase the yield of fish in the sea has been used both experimentally and on a commercial scale in an attempt to increase the fish yield in the sea. Under natural conditions, the sea can only support a limited population of marine animals. The food chain in the sea is very complex, small animals feeding on small plants, larger animals feeding on the small animals, and in turn being fed upon by the fish. Theoretically if the abundance of any link in the chain could be increased, the fish would also increase in quantity. In most marine situations, it does not appear to be feasible to introduce a sufficient number of living organisms to have a significant effect on the food chain. The method which has been used is that of applying chemicals or organic waste to the water to increase the abundance of the lowest living link in the food chain, the tiny plants. If this were done in the open sea an enormous wastage would occur since the ocean currents would disperse the fertilizer and perhaps also the organisms feeding on it. Thus it seems that this experiment could only be successful if conducted in an enclosed area of water. Some water exchange may be necessary to maintain the oxygen supply.

An experiment of this type was carried out in an inlet on the west coast of Scotland. This technique has also been used in pond-culture work in Japan and elsewhere. While an increase in the yield of fish can be obtained using such methods, the gain is not usually in proportion to the amount of fertilizer applied. Some of the fertilizer may be wasted if it sinks to the bottom and became unavailable to living organisms. Some of the fertilizer will be taken up by organisms which do not enter into the food chain of the fish whose yield the experiment is intended to increase. Such organisms may prey upon the organisms in the food chain or on the fish themselves. Hence it is possible, under special circumstances, to increase the yield of marine animals, but "sea farming" will pay only rarely, if at all.

--"Sea Secrets," September 11, 1956
The Marine Laboratory,
University of Miami,
Coral Gables, Fla.

ENZYMES AS AN AID IN SEPARATING OIL FROM PROTEIN IN SALMON EGGS

By Robert M. Kyte*

ABSTRACT

THE DIGESTION OF SALMON EGGS BY ENZYMES OFFERS ADVANTAGES IN SEPARATING OIL FROM PROTEIN IN SALMON EGGS, PARTICULARLY IN ALASKA WHERE THE SOURCE OF EGGS IS WIDELY SCATTERED AND OFTEN IN REMOTE LOCATIONS.

INTRODUCTION

Stansby and Associates (1953A) stated that the oil from salmon eggs was highly unsaturated (iodine value over 200). These authors also found that salmon-egg oil contained significant quantities of cholesterol, although less than reported by earlier workers (Koenig and Grossfeld 1913: Anno 1940), and phospholipids (25-39 percent of the total oil) that might have commercial interest. Research is now being carried out in U. S. Fish and Wildlife Service laboratories and in industrial laboratories on new chemical products (Anonymous 1955) that can be made from fish oil. Kyte (1956) determined the distribution of the fatty acids of which salmon-egg oil is composed and indicated the amount of unsaturation in the fatty acids. The double bonds in the unsaturated salmon egg oil make this oil particularly attractive in the synthesis of polymers and highly substituted compounds and as a reactive intermediate for the synthesis of other organic compounds.

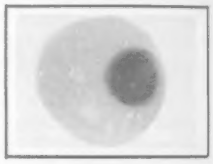

FIG. 1 - PHOTOGRAPH OF SALMON EGG SHOWING OIL DROPLET.

Seagran, Morey, and Dassow (1954) determined the amount of the ten essential amino acids in the protein of salmon eggs. The amount and distribution of these acids indicated salmon-egg protein would be a good animal feed and, being relatively rich in methionine, isoleucine, and lysine, would balance the vegetable protein concentrates which are often deficient in these amino acids. Robinson, Palmer, and Burrows (1951) reported salmon eggs to be an excellent feed for hatchery fish, particularly when water temperatures are over 50° F. and the fish are eating relatively large amounts of food.

Many of the present or potential uses of salmon eggs depend on either the protein fraction or the oil fraction, not the two together. In dried protein concentrates such as fish meals, or in this case egg meal, oil may be an undesirable diluent. Oil for chemical uses or industrial processing is subject to even more rigid purity requirements than is the protein concentrate. Traces of protein matter are undesirable. The purpose of this paper is to discuss methods for separating oil from protein in salmon eggs and, in particular, to stress the role enzymes can play in this process.

METHODS FOR SEPARATING OIL FROM PROTEIN

Sinnhuber (1943) developed a simple method for recovering the highly pigmented oil from the free-oil droplet in salmon eggs (fig. 1). He broke the egg shell or membrane by grinding, then added warm dilute brine (4 percent NaCl) and allowed the oil to separate. The oil was further clarified by centrifuging to remove traces of suspended protein and moisture. This clear brightly-colored oil and the body meat oil derived from the cannery trimmings other than the viscera were used by canners as additives to canned salmon. The Sinnhuber method, however, recovered only one-third of the total oil in the egg and left the protein much diluted. Recovery of the protein was not considered in this method of separating the oil and the process would be expensive.

The dry-rendering process--the drying of fish in a steam-jacketed vessel--is ordinarily used for reducing fish and fish wastes of low oil content to meal. The pressing of the dry-rendered product for removal of oil is usually not carried out because of the difficulty with which the oil is expressed. Salmon eggs processed by this method give a low yield of oil and the protein meal has a very high and undesirable oil content.

*ANALYTICAL CHEMIST, FISHERY PRODUCTS LABORATORY, KETCHIKAN, ALASKA.

The wet-reduction process is used extensively on a commercial scale for the reduction of fish with high oil content such as menhaden and herring. In this process the fish is cooked, then pressed to remove most of the oil and a large portion of the moisture. Laboratory experiments indicated, however, that cooking ground salmon eggs for periods as long as three hours freed only about 65 percent of the available oil (fig.2). The remaining oil was retained with the protein probably bound as a lipo-protein complex. The particle size of the cooked egg protein is very small and pressing this material by itself in the standard fish meal press would be extremely difficult because the cooked protein does not form the necessary mat or shoulder. Thus, neither of the processes most commonly used for the reduction of fish is satisfactory for the handling of salmon eggs.

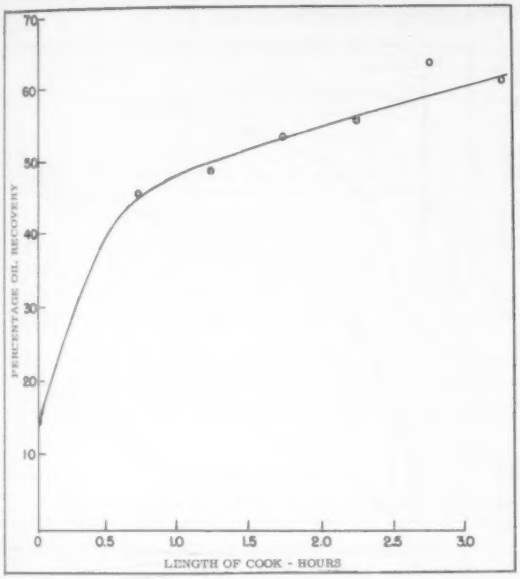

FIG. - 2 THE EFFECT OF COOKING TIME ON OIL RECOVERY FROM PINK SALMON EGGS.

Levin and Lerman (1951) reported an azeotropic distillation process for the dehydration and oil extraction of vegetable and animal products. Tests in this laboratory using an azeotropic distillation with benezene, n-butyl alcohol, or di-chlorethane as solvents for the dehydration and oil extraction or raw fresh salmon eggs, removed from 75 to 80 percent of the oil from the eggs. It was only by the use of several solvents (acetone, ethanol, and a methanol:chloroform mixture) that essentially all of the lipid was removed from the raw salmon eggs.

Chargaff and Cohen (1939) reported the enzymes in rattlesnake venom to be effective in separating certain of the oils in egg yolk. Lovern (1955), however, reported proteolytic enzymes to be ineffective in freeing bound oil from the protein with which it was associated. He reported that the oil was bound quite as strongly to the peptides and other products of enzyme hydrolysis as to the original protein. Nevertheless, proteolytic enzymes might play a desirable role in the separation of oil from protein in salmon eggs.

Thomson, MacLeod, and Idler (1954) report that proteolytic enzymes are used extensively to reduce the viscosity of stickwater in the production of fish solubles. Salmon canneries--the source of supply for salmon eggs--are scattered over a large area and in rather remote locations in Alaska. Preliminary processing at the canneries with minimum equipment and manpower requirements would permit a gross separation of oil and protein and allow the oil and protein concentrates to be collected and transported to centralized processing facilities where efficient utilization might be possible.

EXPERIMENTAL ENZYME DIGESTION

The action of a number of proteolytic enzymes on salmon eggs was investigated. Conditions of pH and temperature reported to be within the active range of the enzymes were maintained and the rates of solubilizing the protein and liberating the oil were observed. The general pattern of action was the formation of a clear protein solution, a free oil, an oil emulsion layer, and, in some cases, a small amount of

sediment. Figure 3 shows the rate of formation of clear solution from salmon eggs using pepsin (N. F. grade), Rhozyme B-6 and Rhozyme A-4 (commercial proteolytic enzymes) and Bromelain (a mixture of several proteolytic enzymes obtained from the stem of the pineapple plant). Bacterial action was inhibited by the addition of 20 p.p.m. of aureomycin (chlortetracycline hydrochloride).

Even though the protein solution of the enzyme digest was clear after centrifuging, 10 to 20 percent of the dry weight of the protein solution was oil (as determined by acid hydrolysis). A benzene or dichloroethane azeotropic distillation of the enzyme digest removed one-half to two-thirds of the oil but the dried residue still contained 6 to 8 percent oil. The enzyme digestion did, however, solubilize or put into solution the egg protein and did permit a gross separation of an oil emulsion and protein solution by simple settling or centrifuging.

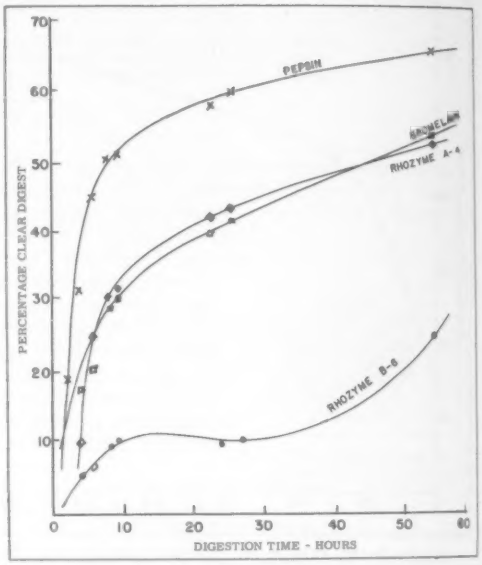

FIG. 3 - THE EFFECT OF DIGESTION TIME ON THE AMOUNT OF CLEAR DIGEST FORMED IN THE DIGESTION OF SALMON EGGS USING ONE PERCENT BY WEIGHT OF FOUR DIFFERENT ENZYMES.

The oil emulsion phase contained 40 to 60 percent oil and 5 percent protein. The dried protein residue from the enzyme digestion had a bright orange glassy to dull semiplastic appearance depending on the enzyme and length of digestion.

The oil of the salmon egg was recovered as free oil and oil emulsion on centrifuging or allowing the digest to settle. The emulsion was very stable. The oil could be recovered from the emulsion, however, by drying or solvent extraction. The oil recovered from the emulsion was bright red, and was similar in appearance to brine-extracted oil. However, its chemical characteristics were not determined.

The digestive tract of fish contains a variety of proteolytic enzymes (Stansby and Associates 1953B: Kenyon 1925) which probably attack peptides and peptones (protein fragments) as well as whole proteins. The enzyme system in the digestive tract of pink salmon was effective in solubilizing the salmon egg protein (a direct comparison of rate with the other enzymes was not obtained) and permitted an azeotropic distillation extraction to remove essentially all of the oil from the protein residue. It is suggested that the enzyme system of salmon digestive tracts either broke the salmon egg protein molecule to very short fragments from which the lipid could be readily extracted or contained an enzyme which split the protein lipid bond.

NUTRITIVE QUALITY OF ENZYME DIGEST

The effect of enzyme digestion on the nutritive value of the salmon egg protein had not been established. A single evaluation of three samples of dried salmon egg protein was carried out by Dr. Grau of the University of California by a method found effective in evaluating fish protein in poultry rations (Grau 1955). The protein was prepared from (a) salmon eggs with fat removed by exhaustive solvent extraction, (b) salmon eggs digested with the enzymes of the digestive tract of salmon and solvent extracted to remove the fat, and (c) salmon eggs digested with the commercial enzyme Rhozyme B-6 and not solvent extracted. The results are shown in table 1 and indicate that, in chick feeding, enzyme digestion did not affect the protein quality.

Table 1 - Quality of the Protein of Salmon-Egg Meals Produced With or Without Enzyme Digestion as Determined by Chick Feeding Tests

	Sample	Gain/Day	Gain/Feed
		. . . . (Percent). . . .	
Salmon-Egg Meal	Defatted by exhaustive solvent extraction	4.8	0.42
	Enzyme digested 1/, solvent defatted	4.9	0.38
	Enzyme digested 1/, high fat content	4.8	0.42

1/ENZYME USED WAS THE DIGESTIVE TRACT OF A PINK SALMON, ONE PERCENT OF THE WEIGHT OF THE EGGS.
2/ENZYME USED WAS RHOZYME B-6 AT 0.5 PERCENT WEIGHT OF THE EGGS.

With the salmon egg-enzyme systems investigated, bacterial decomposition became a factor of major importance in the pH range of 4.5 to 8.5. Aureomycin (chlortetracycline hydrochloride) at 20 p.p.m. was found effective in controlling bacterial action for over one week. Twelve p.p.m. aureomycin did not adequately inhibit bacterial action as judged by the formation of offensive odors.

Preliminary experiments with the complete offal from the canning of salmon indicated that enzymes were effective in dissolving most of the protein and separating a large part of the oil from the proteins.

SUMMARY

The oil and protein contained in salmon eggs are valuable when separated from each other. Common methods of reduction are not entirely satisfactory for separating oil from protein in salmon eggs.

The action of certain enzymes on salmon eggs partially separates the oil from protein as it solubilizes the protein.

An enzyme digestion might find application to salmon cannery offal as well as to salmon eggs as a processing step in producing concentrates of oil and protein at scattered and remote canneries in Alaska.

ACKNOWLEDGMENT

The author is indebted to Rohm and Haas Company for samples of Rhozyme A-4 and Rhozyme B-6; Libby, McNeill and Libby Company for samples of Bromelain; and the Cudahy Packing Company for samples of pepsin.

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DOMESTIC SOAP FROM SEAL OIL

In localities where seal oil is easily available it would probably be advantageous if it could be used in the form of domestic soap. But raw seal oil contains unsaturated molecules which are responsible for the softness of the soap and its tendency to turn rancid rapidly. In order to obtain a hard, stable soap, it would therefore be necessary to change the unsaturated condition of the oil. This could be accomplished by hydrogenation, a process which is used mostly for edible oils; but because of the special apparatus required, it is not adaptable to home use. It was thought that polymerization, which can be carried out at home, might furnish a solution to the problem.

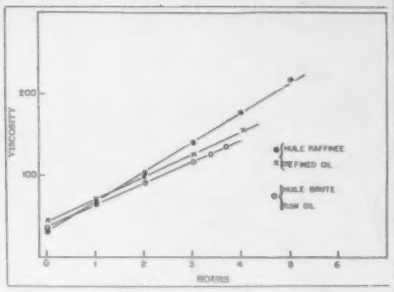

Fig. 1 - Variation in iodine value with time of polymerization.

Polymerization makes the unsaturated molecules react among themselves; the extent of which depends upon the temperature used and the length of time the reaction is allowed to proceed.

In a previous study, it was established that polymerization of seal oil proceeds readily at a temperature of 525° F. However, the speed of the reaction in an open air vessel was unknown. Therefore, several experiments were carried out to follow the course of the reaction over a period of hours. Since polymerization produces a lowering in iodine value, and an increase in viscosity, the iodine value and viscosity of seal oil heated at 525° F. were followed on samples withdrawn at hourly intervals. Figure 1 shows the rate at which the iodine value decreases during the polymerization of raw and of refined seal oil. It also shows that the reaction is at first rapid and that it then slows down gradually, the rate being the same for refined and unrefined oil.

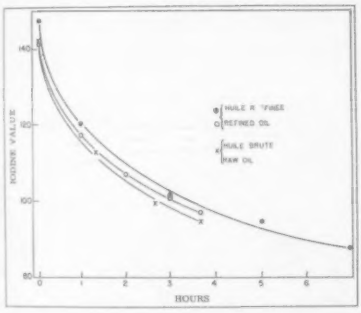

Fig. 2 - Viscosity change with time of polymerization.

The iodine value of seal oil is about 145. It must be lowered to at least 90 before the oil shows resistance to oxidation. Thus, it is apparent that the oil must be heated for about four hours to obtain the desired iodine value.

Figure 2 shows the change in viscosity which accompanies polymerization. It shows that the viscosity of the oil increases regularly with time. In practice, the viscosity index could be used to determine the extent of polymerization and thus to indicate whether uniform products were obtained.

Since the object of this investigation was the preparation of soap, some oil was first heated at 525° F. for four hours, then subjected to the usual procedure for the preparation of domestic soap. A product of suitable firmness was obtained. With the addition of rosin, it showed good lathering properties.

Thus the polymerization process offers a cheap means of preparing domestic soap from seal oil. No special apparatus is required since polymerization and saponification can be done in the same kettle. The technique is also very simple: it consists in heating the oil at 525° F. for about four hours. After polymerization the oil is transformed into soap by means of the standard procedure used for the preparation of domestic soap.

At 525° F. the oil gives off smoke and care must be taken not to heat it much higher because it might catch fire.

The process described above could, in all probability, be applicable to other oils, such as fish oils, to yield a domestic soap of fair quality. Depending on the kind of oil used, certain modifications of the temperature and heating time would no doubt be required.

"Gaspe Fisheries Experimental Station, Note No. 35"
Progress Reports of the Atlantic Coast Stations, Aug. 1954
Fisheries Research Board of Canada

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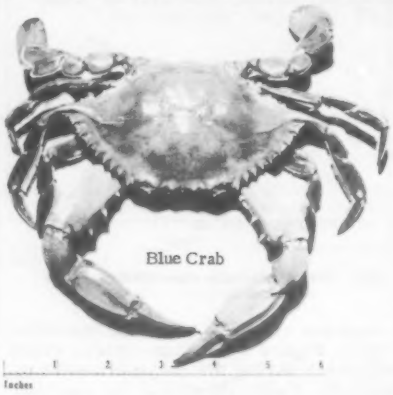
BOOKLET ON "HOW TO COOK CRABS"

Housewives, home economists, and food editors will be interested in a new fish-cookery publication, How to Cook Crabs, which was released January 24 by the United States Fish and Wildlife Service. The booklet contains 32 choice recipes which have been developed and kitchen-tested by the Service's staff of home economists.



Crabs are one of our popular shellfish because of their tender meat and distinctive flavor. Crab meat also is an excellent source of high-quality proteins, vitamins, and minerals needed for good nutrition. Modern processing and marketing methods now make crabs available almost everywhere in the United States. Crabs are available in these market forms: live; cooked in the shell; cooked and frozen; fresh cooked meat; and canned meat.

As the booklet explains, four principal kinds of crabs are taken from the marine waters of the United States and Alaska. From the Atlantic and Gulf coasts come blue crabs which compose three-fourths of all the crabs marketed in this country. Dungeness crabs are found on the Pacific coast from Alaska to Mexico. King crabs come from the North Pacific off Alaska. Rock crabs are taken on the New England and California coasts. Of local importance are stone crabs in Florida and tanner crabs in Alaska.

The four principal kinds of crabs are pictured in the booklet and their approximate weights are listed. Complete illustrated instructions are given for picking the meat from blue crabs.

Some of the easy-to prepare recipes included in the new publication are: Crab Louis, Crab Ravigote, Crab Newburg, Deviled Crab, Imperial Crab, Avocados Stuffed with Crab Meat, and Barbecued Crab Sandwiches.

Generously illustrated, How to Cook Crabs, Test Kitchen Series No. 10, may be purchased for 20 cents each from the Superintendent of Documents, Government Printing Office, Washington 25, D. C.